

TIMING AND INTENSITY OF FLOWERING OF SWEET ORANGE [*Citrus sinensis*
(L.) Osbeck] AS A FUNCTION OF LOCAL WEATHER FACTORS AND CROP
UNDER CENTRAL FLORIDA CONDITIONS



By

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I would like to dedicate this work to my mother Guadalupe Banuet

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TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS.....	iv
LIST OF TABLES	x
LIST OF FIGURES.....	xiv
ABSTRACT	xiv
CHAPTER	
1. INTRODUCTION.....	1
Overview of Citrus Flowering.....	1
Flower Bud Induction.....	2
Flower Bud Differentiation	3
Flower Opening.....	3
Factors Affecting Flowering in Citrus	4
Exogenous Factors	5
Ambient temperature.....	5
Soil temperature	6
Water relations	6
Endogenous Factors	7
Plant material.....	7
Plant age	7
Carbohydrates.....	8
Hormones and growth regulators	9
Plant nutrition.....	12
Bud and shoot age	14
Crop load	15
In Vitro Flowering.....	16
Phenology Studies of Citrus Flowering	17
Modeling of Agricultural Systems	17
Expert Systems and Decision Support Systems.....	18
Modeling Phenological Processes.....	19
Modeling Citrus Flowering.....	20

2. EFFECT OF LOW TEMPERATURE STRESS LEVELS, CROP LOAD, AND BUD AGE ON THE FLORAL INDUCTION OF SWEET ORANGE TREES [<i>Citrus sinensis</i> (L.) Osbeck] IN CENTRAL FLORIDA	23
Flower Bud Induction.....	24
Monitoring Flower Bud Induction	25
Effect of Ambient Temperatures.....	26
Effect of Crop Load.....	27
Effect of Bud Age	28
Materials and Methods.....	29
Plant Materials.....	29
Experimental Design	30
Data Collection.....	31
Analysis 1. Phenological Response of 'Valencia' and 'Hamlin' Trees as Affected by Season and Crop.....	33
Effect of season and crop load on bud sprouting parameters.....	33
Effect of season and crop load on flowering phenology	34
Floral distribution by shoot age (summer vs. spring).....	34
Analysis 2. Effect of Different Induction Levels on the Phenological Response of 'Valencia' and 'Hamlin' Trees.....	34
Effects of different induction and crop load treatments on phenological parameters	34
Effects of different induction levels on floral distribution by bud position along the shoot	35
Analysis 3. Effect of Weather Data on Phenological Response of 'Valencia' and 'Hamlin' Trees	35
Induction as a function of weather	35
Weather data.....	36
Phenological response	36
Results	36
Analysis 1. Phenological Response of 'Valencia' and 'Hamlin' Trees as Affected by Season and Crop.....	36
Effect of season and crop load on bud sprouting	36
Effect of season and crop load on flowering phenology	37
Floral distribution by shoot age (summer vs. spring).....	38
Analysis 2. Effect of Different Induction Levels on the Phenological Response of 'Valencia' and 'Hamlin' Trees.....	39
Effects of different induction levels and crop load on phenological parameters ..	39
Effects of different induction levels on floral distribution by bud position along the shoot	40
Analysis 3. Effect of Weather Data on the Phenological Response of 'Valencia' and 'Hamlin' Trees.....	41
Vegetative buds per shoot	41
Flowering buds per shoot.....	41
Flowers per flowering bud	41
Flowers per shoot	42
Discussion	42

Analysis 1. Evaluation of Phenological Response (Bud Sprouting and Flowering Parameters) of 'Valencia' and 'Hamlin' Trees as Affected by Season and Crop Load.....	42
Bud sprouting parameters.....	42
Effect of season and crop load on flowering phenology.....	43
Floral distribution by shoot age (summer vs. spring).....	44
Analysis 2. Effect of Different Induction Levels on the Phenological Response of 'Valencia' and 'Hamlin' Trees.....	45
Effects of different induction levels and crop load on phenological parameters..	45
Effects of different induction levels on the floral distribution by bud position along the shoot	47
Analysis 3. Effect of Weather on the Phenological Response of 'Valencia' and 'Hamlin' Trees	47
Vegetative buds per shoot	47
Flowering buds per shoot	48
Flowers per flowering bud	49
Flowers per shoot.	50
Conclusions	50
 3. MODELING FLOWERING DATE OF SWEET ORANGE [<i>Citrus sinensis</i> (L.) Osbeck] TREES IN CENTRAL FLORIDA BASED ON HISTORICAL WEATHER RECORDS	82
Materials and Methods	83
Analysis 1. Flowering Date as a Function of Average Monthly Temperatures.....	85
Analysis 2. Flowering Date as a Function of Hours Accumulative Within Monthly Temperature Ranges.....	86
Analysis 3. Days to Full Bloom as a Function of Stratified Degree-Day Analysis after Fulfillment of Induction Stimuli and Initiation of Differentiation.....	86
Analysis 4. Days to Full Bloom as a Function of Phenological Parameters After Fulfillment of Induction Stimuli and Initiation of Differentiation	87
Determining differentiation time after fulfillment of induction stimuli and initiation of differentiation	88
Determining date of initiation of differentiation based on the reported date for peak anthesis	88
Results	89
Analysis 1. Flowering Date as a Function of Average Monthly Temperatures.....	89
Analysis 2. Flowering Date as a Function of Hours Accumulated Within Monthly Temperature Ranges.....	89
Analysis 3. Days to Full Bloom as a Function of Stratified Degree-day Analysis after Fulfillment of Induction Stimuli and Initiation of Differentiation.....	90
Analysis 4. Days to Full Bloom as a Function of Phenological Parameters after Fulfillment of Induction Stimuli and Initiation of Differentiation.	91
Determining differentiation time after fulfillment of induction stimuli and initiation of differentiation	91
Determining date of initiation of differentiation based on the reported date for peak anthesis	92

Discussion	92
Conclusions	97
 4. INTEGRATION CHAPTER. DECISION SUPPORT SYSTEM FOR THE PREDICTION, AND MANAGEMENT OF THE FLOWERING PROCESS OF SWEET ORANGE TREES [<i>Citrus sinensis</i> (L.) Osbeck] UNDER FLORIDA CONDITIONS	110
Materials and Methods	112
Overall Framework of the DISC-DSS	112
Induction Monitoring Expert System	112
Transition from Induction to Differentiation Models	114
Models for Floral Organ Differentiation – Time to Bloom Models	115
Results	116
Overall Framework of the DISC-DSS	116
Induction Monitoring Expert System	117
Transition from Induction to Differentiation Models	121
Models for Floral Organ Differentiation – Time to Bloom Models	121
Discussion and Conclusions	123
 APPENDIX A. DEVELOPMENT OF FLOWERING BUDS IN EXCISED STEMS OF SWEET ORANGE [<i>Citrus sinensis</i> , (L.) Osbeck]	133
Materials and Methods	136
Experiment 1. Preliminary Studies	136
Experiment 2. Forcing Technique for the Development of Flowers in Previously Induced Buds	136
Collection of plant materials	137
Shoot preparation	137
Shoot forcing	137
Results	138
Experiment 1. Preliminary Studies	138
Experiment 2. Forcing Technique for the Development of Flowers in Previously Induced Buds	138
Discussion	138
Conclusions	140
 LIST OF REFERENCES	143
 BIOGRAPHICAL SKETCH	149

LIST OF TABLES

<u>Table</u>	<u>Page</u>
2-1 Description of induction and crop load treatments (6 reps.) of sweet orange trees for Valencia and Hamlin during the 1998-1999 and 1999-2000 seasons	53
2-2. Comparison of bud sprouting parameters of 'Hamlin' sweet orange trees from the 1999 to the 2000 seasons (rows), and with and without crop load (columns)	54
2-3. Comparison of bud sprouting parameters of 'Valencia' sweet orange trees from the 1999 to the 2000 seasons (rows), and with and without crop load (columns)	55
2-4. Comparison of flowering parameters of 'Hamlin' sweet orange trees from the 1999 to the 2000 seasons (rows), and with and without crop load (columns)	56
2-5. Comparison of flowering parameters of 'Valencia' sweet orange trees from the 1999 to the 2000 seasons (rows), and with and without crop load (columns)	57
2-6. Cumulative percentages of floral distribution by bud position along the shoot of different age shoots (summer and spring) for 'Hamlin' and 'Valencia' trees combining the 1999 and 2000 seasons.....	59
2-7. Summary of analyses of variance of bud sprouting parameters (total sprouting, vegetative, and reproductive buds per shoot) for 'Hamlin' and 'Valencia' trees during the 1999 and 2000 seasons.....	60
2-8. Summary of sprouting parameters (vegetative and reproductive buds per shoot) for 'Hamlin' trees during the 1999 season.....	61
2-9. Summary of sprouting parameters (vegetative and reproductive buds per shoot) for 'Hamlin' trees during the 2000 season.....	62
2-10. Summary of sprouting parameters (vegetative and reproductive buds per shoot) for 'Valencia' trees during the 1999 season.....	63
2-11. Summary of 'Duncan' analyses of sprouting parameters (vegetative and reproductive buds per shoot) for 'Valencia' trees during the 2000 season.	64

2-12. Summary of analyses of variance of flowering parameters (number of flowers per shoot, number of flowers per flowering bud, and number of flowers per sprouting bud) for 'Hamlin' and 'Valencia' trees during the 1999 and 2000 seasons.....	65
2-13. Summary of flowering parameters (total number of flowers per shoot, total number of flowers per flowering bud, and total number of flowers per sprouting bud) for Hamlin trees during the 1999 season.....	66
2-14. Summary of flowering parameters (flowers per shoot and number of flowers per flowering bud) for 'Hamlin' trees during the 2000 season.	67
2-15. Summary of flowering parameters (total number of flowers per shoot, total number of flowers per flowering bud, and total number of flowers per sprouting bud) for 'Valencia' trees during the 1999 season.....	68
2-16. Summary of flowering parameters (total number of flowers per shoot, total number of flowers per flowering bud, and total number of flowers per sprouting bud) for 'Valencia' trees during the 2000 season.....	69
2-17. Summary of analyses of variance for the effect of induction and crop load treatments on the development of flowers per shoot for 'Hamlin' and 'Valencia' trees during the 1999 and 2000 seasons	70
2-18. Summary of number of flowers per shoot by position along the shoot of Hamlin branches during the 1999 season.....	71
2-19. Summary of number of flowers per shoot by position along the shoot of Hamlin branches during the 2000 season.....	72
2-20. Summary of number of flowers per shoot by position along the shoot of Valencia branches during the 1999 season.....	73
2-21. Summary of number of flowers per shoot by position along the shoot of Valencia branches during the 2000 season.....	74
2-22 Results of stepwise multiple regression analyses relating the number of vegetative buds per shoot (dependent variable) to temperature data in the form of the total number of hours in ranges of 5°C increments, average temperature, and crop load status (independent variables) for the combined data of the 1999 and 2000 seasons.....	77
2-23. Results of stepwise multiple regression analyses relating the number of flowering buds per shoot (dependent variable) to temperature data in the form of the total number of hours in ranges of 5°C increments, average temperature, and crop load status (independent variables) for the combined data of the 1999 and the 2000 seasons.....	78

2-24. Results of stepwise multiple regression analyses relating the number of flowers per flowering bud (dependent variable) to temperature data in the form of the total number of hours in ranges of 5°C increments, average temperature, and crop load status (independent variables) for the combined data of the 1999 and the 2000 seasons.....	79
2-25. Results of stepwise multiple regression analyses relating the total number of flowers per shoot (dependent variable) to temperature data in the form of the total number of hours in ranges of 5°C increments, average temperature, and crop load status (independent variables) for the combined data of the 1999 and the 2000 seasons.....	80
2-26. Summary of weather data for Lake Alfred, Florida during the 1998-1999 and 1999-2000 seasons.....	81
3-1. Data records for 20 years of flowering dates for oranges in Central Florida in which a single bloom period was detected.	99
3-2. Induction monitoring expert system rule database set for the determination of induction status.....	100
3-3. Phenological parameters from reported data in the literature.	101
3-4. The association of date of flowering of orange trees with significant monthly mean temperatures by stepwise multiple regression analysis in Florida for 40 years (1959-1998).	103
3-5. The association of date of flowering with significant monthly hours of stratified temperatures for oranges in Central Florida for a 40-year period (1959-1998). Stepwise multiple regression analysis using temperature records (November to March of each year) stratified into five temperature ranges (below 5, 5 to 12.8, 12.8 to 20, 20 to 30, and above 30°C) was performed.	104
3-6. Stepwise multiple regression analysis of days to peak anthesis to degree-days ((hourly temperature - threshold) $\times 24^{-1}$) for different temperature ranges. The best degree-day ranges for this analysis to maximize accounting for variability were: below 12.8°C, from 12.8 to 20.8°C, and above 20.8°C. Initiation of differentiation was determined manually by examining the occurrence of warm periods after mid-December.....	105
3-7. Comparison of observed vs. predicted days to full bloom based on degree-day analysis.	106
3-8. Transformation of equation from 3-6 into percent of floral development.....	107
3-9. Total percentage of floral development from initiation to peak anthesis based on the floral development equation.	108

3-10. Comparison of the estimated initiation time based on the floral development equation and after a warm period was considered to have initiated differentiation. A comparison of total inductive hours before each alternative initiation time is presented.	109
4-1. Induction monitoring expert system rule database set for the determination of induction status.....	125
4-2. Expert System rule database set to delay the initiation of differentiation at low induction levels	126
4-3. Expert System rule database set to delay the initiation of differentiation at moderate induction levels	127
4-4. Expert System rule database for the enhancement of flower bud induction.....	128
4-5. Expert System rule database for the reduction of induction levels.....	129
4-6. Expert System rule database to decrease the intensity of secondary blooms.....	130
4-7. Expert System rule database to reduce induction levels and decrease flowering intensity	131
4-8. Summary of El Niño and La Niña years from 1958-1959 to 1999-2000. Years not mentioned are considered neutral.....	132
A-1. Summary of observed response of citrus buds with two different levels of floral induction to different concentrations of cytokinins and sucrose	142

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
2-1. Local temperature data from the Florida Automated Weather Network system for the Lake Alfred station during the 1998-1999 and 1999-2000 seasons.	52
2-2. Distribution of flowers by shoot age (summer vs. spring shoots).....	58
2-3. Effects of different induction levels and crop load treatments on the distribution of flowers by bud position along the shoot of 'Hamlin' sweet orange trees during the 1999 and 2000 seasons. Data are presented as flowers per shoot.	75
2-4. Effects of different induction levels and crop load treatments on the distribution of flowers by bud position along the shoot of 'Valencia' sweet orange trees during the 1999 and 2000 seasons. Data are presented as flowers per shoot.	76
3-2. Floral development equation. The association of average daily temperatures with days to full bloom based on reported differentiation time. If the average daily temperature was assumed to cause flowering to occur in n days then the corresponding development fraction for that day was n^{-1}	102

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Under Florida conditions, flower bud induction is caused by low-temperature stress during the winter. High temperatures during spring bring about inflorescence differentiation, followed by anthesis. A study was conducted to quantify the flowering response of citrus trees to low temperatures and crop load. Selected branches of mature sweet orange 'Valencia' and 'Hamlin' trees, carrying two levels of crop load, were tagged and then exposed to fall-winter inductive conditions. Floral induction was interrupted during the wintertime (November to February) twice monthly of different sets of branches. Treatments consisted of different flower induction levels. Phenological response to season, induction, and crop load treatments was evaluated in terms of bud sprouting and flowering response (including floral distribution). Phenological response was related to weather information using regression analysis. The factors that determined the phenological response of an individual bud to environmental conditions included

cultivar, position of the bud along the shoot, shoot age, induction level, and crop load. Hours in the high temperature ranges decreased the sprouting of vegetative buds, while low temperatures increased the sprouting of reproductive buds. Crop load decreased the sprouting of reproductive buds and increased the sprouting of vegetative buds. Low temperatures and crop load were the two most important factors affecting floral intensity.

A second study was conducted that analyzed the effect of Florida's irregular winter weather on date of peak flowering, which may vary from mid-February to mid-April. Historical weather and flowering records (from 1959 to 2000) were used to develop mathematical models to predict the occurrence of peak anthesis and explain the presence of multiple bloom periods. Models that use average temperature, number of hours within temperature ranges, and degree-days were established to predict bloom date. These models, although accurate under Florida conditions, may not be used in other citrus production areas. Periods of relatively high temperatures occur during the winter and spring in Florida. Furthermore, insufficient high temperatures during differentiation and flower bud expansion also prevent extrapolation to tropical environments.

A Decision Support System (DSS) was developed to provide growers with recommendations for their citrus production practices. Best management practices often require that a given practice be applied at a specific stage of phenological development. Because of the variable flowering dates that occur, the proper timing of any given production practice may vary by as much as 2 months. The DSS includes modules for phenological development and for the scheduling of production practices. Mathematical models developed from this research were implemented to drive the phenology component. The phenological component was integrated with other expert systems, to aid in the decision making process of citrus growers.

CHAPTER 1 INTRODUCTION

Citrus is produced commercially between latitudes 40°N and S. Within these latitudes several important climatic characteristics affect the growth and development of citrus trees. The effect of climate is particularly important for flowering and fruiting of citrus. The objective of this review is to present an overview of citrus flowering, a discussion of the factors that affect it, and an overview of the phenological models developed to explain and predict citrus flowering.

Overview of Citrus Flowering

Flowering in perennials is more complex than in herbaceous annuals. In herbaceous annuals, flower bud induction results in the conversion of all meristems to the flowering state. In citrus, as in all perennials, some buds are induced into reproductive growth while others remain committed to vegetative growth (Lord and Eckard, 1985).

Flowering in citrus consists of induction and differentiation periods preceding anthesis. Induction refers to the triggering of the flowering process by environmental factors resulting in a change in the developmental pattern of the buds, which then become committed to flowering (Guardiola, 1997). Subsequent differentiation involves histological and morphological changes in the vegetative meristem that becomes a floral meristem (Davenport, 1990).

Flower Bud Induction

Induction is the mechanism that results in the commitment of meristematic cells to the formation of reproductive structures. Induction is an activating or derepressing mechanism of the flowering process brought about by environmental factors. The predominant flower bud induction stimulus varies according to the environmental conditions to which the trees are exposed. Flowering in citrus is considered day-neutral and self-inductive with no single, indispensable induction stimulus known (Moss, 1969). Climatic conditions play a significant role in the vegetative and reproductive phases of citrus. Citrus trees growing in tropical conditions flower repeatedly throughout the year with peak levels occurring when rainfall follows periods of dry weather. Under these conditions, flowers are induced during the water-stress period, but the beginning of reproductive growth does not occur until the onset of the rainy period (Cassin et al., 1969).

Under subtropical conditions, citrus trees produce flowers primarily during the spring after the winter season (García-Luis et al., 1995a; Simanton, 1969). Induction occurs during the winter-rest period as inhibitors applied at this time inhibit flower induction completely (Monselise and Halevy, 1964). The first step toward induction of flowers is a cessation of vegetative growth caused by low temperatures. Low temperatures also cause the buds to remain inactive during the winter season, when they are induced to flower (Reuther et al., 1973). The release of the stress by higher temperatures signals the beginning of reproductive growth (Cassin et al., 1969).

Water and low-temperature stresses cause flower bud induction under tropical and subtropical conditions, respectively. The duration, intensity, and time course of the stresses directly influence timing and intensity of citrus flowering (Furr and Armstrong,

1956; Ginestar and Castel, 1996). Flower bud induction from these two stresses is not mediated through a common decrease in xylem pressure potential (Southwick and Davenport, 1986). Although no conclusive explanation has been provided for the similarity in flowering response from water and low-temperature stress, the possible role of roots signaling to start bud break and flowering has been proposed (Poerwanto and Inoue, 1990).

Flower Bud Differentiation

Differentiation involves the histological and morphological changes in the vegetative meristem as it becomes a floral meristem (Davenport, 1990). Vegetative and reproductive meristems are anatomically indistinguishable even after full induction. The differentiation of the floral organs does not occur until the first stages of bud swelling. Under subtropical conditions, the onset of differentiation (initiation) takes place in spring. Initiation date varies in different citrus cultivars. Initiation can vary even within branches in the same tree, with the southeastern part of the tree being the first to sprout, and the northwestern part of the tree the last one (Abbott, 1935; Lomas and Burd, 1983; Randhawa and Dinsa, 1947). The morphological development of the flowers follows the initiation of the flower primordia. The pattern of organ differentiation and its morphological changes during floral and inflorescence development have been described (Altman and Goren, 1978; Lord and Eckard, 1985). The rate of development depends on temperature and flowering intensity (Guardiola, 1997; Moss, 1973).

Flower Opening

Flower opening (anthesis) occurs after the induction and differentiation processes have been completed, usually when favorable temperature and/or water-relations exist. Time to bloom and duration of flower opening are temperature dependent (Bellows and

Morse, 1986; Lovatt et al., 1984; Simanton, 1969). Higher temperatures shorten the time to reach anthesis (Lomas and Burd, 1983).

In subtropical areas, all flowers of a tree do not reach anthesis simultaneously. There is variation in flowering date and intensity according to bud position, inflorescence type, and floral position. The normal sequence of anthesis within an inflorescence is apical flower first, then basal flower, then the subapical flower. Early seasonal flowering tends to be associated with a greater number of inflorescences than late seasonal flowering. However, late inflorescences are more likely to have a greater number of leaves than early inflorescences (Jahn, 1973). Flowering may also vary depending on the weather and the successive satisfaction of the induction requirement. Flowering may occur successively in different buds or branches on a tree depending on the satisfaction of the induction requirement (Chapters 2 and 3).

The intensity of flowering depends on a number of factors including duration and intensity of the stress (Lovatt et al., 1988a). A positive correlation exists between the duration of the low-temperature treatment and the number of floral shoots initiated by 'Tahiti' lime trees (*Citrus latifolia* Tan.) (Southwick and Davenport, 1986). Similarly, induction at higher temperatures result in the formation of fewer flowers associated with a greater vegetative component (Moss, 1969).

Factors Affecting Flowering in Citrus

A number of exogenous and endogenous factors are known to influence citrus flowering. The main exogenous factors known to affect flowering behavior include water relations, and both air and soil temperatures. Endogenous factors include cultivar, tree age, carbohydrate status, phytohormones, nutrition, crop load, and bud/shoot age.

Exogenous Factors

Ambient temperature

Ambient or air temperature is the single most important factor affecting floral induction and development in subtropical environments. Low temperatures are the primary environmental condition leading to flower bud induction. In addition, low temperatures seem to favor bud sprouting. The inductive effect of low temperature is followed by flower differentiation, which is related to the seasonal temperature increases in spring (Cassin et al., 1969; García-Luis et al., 1992).

Air temperature affects flowering date, morphological characteristics, and flowering intensity. Flowering date depends on 1) the length and intensity of the temperature inductive conditions, and 2) the temperatures during differentiation period. During normal winter seasons, a long winter will produce a flowering period later than the average (Lomas and Burd, 1983). In addition, high day temperatures during floral organ differentiation would reduce flower development time and therefore advance date of anthesis (Moss, 1973). Ambient temperatures also influence morphological characteristics of flowers and ovaries. High ambient temperatures are associated with smaller flowers with small but long ovaries (Inoue, 1989). At low air temperatures, flowers and ovaries are bigger than at higher temperatures. As for flower intensity, high temperatures during bud sprouting are reported to increase the leafiness of the inflorescences and to inhibit flowering. By contrast, number of flowers per tree, number of flowers per node, and sprouting rate are greater at low air temperatures (Poerwanto and Inoue, 1990).

Soil temperature

Soil temperature influences both flower bud induction and differentiation. During the induction period, low soil temperatures are reported to induce flowers in satsuma mandarin (Poerwanto et al., 1989). However, if the induction requirement has been satisfied, soil temperature does not result in additional induction or in significant effects on flower morphology (Poerwanto and Inoue, 1990). At bud break, high soil temperatures increase bud sprouting, but have little effect on flower number (Hall et al., 1977). During floral differentiation high soil temperature did not inhibit flower development (Moss, 1976).

Soil temperature effects on flower morphology during flower bud induction. Low soil temperatures combined with high day air temperatures inhibit floral organ differentiation, especially of the ovary, and lead to some malformed flowers (Taniguchi, 1981). At low soil temperatures, flowers and ovaries were bigger than at the higher soil temperature. Changes in soil temperature do not result in significant effects on flower morphology, as long as these changes occur after the inductive requirement is satisfied (Poerwanto and Inoue, 1990).

The enhancement of flowering by high soil temperatures indicates an active role of the root system on the flowering process. High soil temperature may influence flowering either by enhancing hormonal export from the roots, or by increasing nutrient and/or water uptake, which in turn produces more adequate conditions for floral bud differentiation (Poerwanto and Inoue, 1990).

Water relations

Soil water content has a direct effect on citrus flowering, especially under tropical conditions. Citrus trees growing in tropical areas have flower buds induced primarily by

the effects of drought stress. The duration and level of the water stress directly influences flower intensity (Ginestar and Castel, 1996). Flower bud development does not occur until after rainfall or irrigation releases water stress (Cassin et al., 1969; Southwick and Davenport, 1986).

Soil water availability also has an effect under subtropical conditions. Low soil water content during the release of low temperature stress diminishes bud sprouting and floral development. In addition, a reduction in soil water content during anthesis markedly decreases flower viability (González-Altozano and Castel, 1999).

Endogenous Factors

Plant material

The flowering response varies among citrus cultivars and by the effect of rootstocks. In Florida, the precise timing of initiation of differentiation can vary by up to 3 weeks depending on citrus cultivars (Abbott, 1935). The rate of floral development and the intensity of flowering are also dependent on citrus cultivar (Randhawa and Dinsa, 1947).

Plant age

Juvenility is the primary effect of plant age on flowering. Juvenility is a process that occurs in all angiosperms by which flowering does not occur regardless of the presence of the inductive stimulus (Chailakhan, 1968). Citrus seedlings flower for the first time at 5 to 10 years of age. Differences in the length of the juvenile period vary according to cultivar: tangerines (*Citrus reticulata* Blanco) 5 to 7 years, sweet orange [*Citrus sinensis* (L.) Osbeck] 6 to 7 years, grapefruit (*Citrus paradisi* Macf.) 7 to 8 years, tangelo (*Citrus paradisi* Macf. \times *Citrus reticulata* Blanco) 5 to 8 years. In addition plant parts within the same plant may present different juvenile characteristics at a given time.

The buds farthest from the root produce shoots that are the least juvenile in character, and those nearest to the root produce shoots most juvenile in character (Furr et al., 1947).

Carbohydrates

Carbohydrate status of the tree influences the number of floral shoots and total number of flowers initiated (Lovatt et al., 1988a). Although a relationship between carbohydrate levels and flowering has been inferred, carbohydrates (starch) are not considered to influence flower differentiation directly, but serve as substrates for the synthesis of metabolites that act alone or through plant hormones at the genetic level to initiate flower bud differentiation. In addition, the accumulation of nonstructural carbohydrates from bud sprouting to anthesis (in leaves and other tree parts) indicates that photoassimilates exceed carbohydrate consumption (Sanz et al., 1987). Thus, the proposition that the levels of carbohydrates may be the limiting factor for flower development (Goldschmidt and Golomb, 1982) seems unlikely (García-Luis et al., 1995a; García-Luis et al., 1992; Sanz et al., 1987).

Manipulations that cause an increase in carbohydrate content quantitatively increase flower intensity in the following season (Agusti et al., 1992). Fall girdling (ringing) promotes the formation of leafless inflorescences and inhibits vegetative growth (Erner, 1988; Iwahori et al., 1990). Other studies found that a reduction in crop load before winter, either through thinning part of the crop or through the advancement of harvest also increases flower intensity during the following season (García-Luis et al., 1995a; García-Luis et al., 1988). Carbohydrates alone were discarded as the main regulatory factor for the effect of girdling (or crop load) on flower formation (García-Luis et al., 1995a).

Although flower buds are able to fix CO₂ into a number of compounds in their own tissues, they are primarily considered heterotroph organs (Vu et al., 1985). During profuse flowering, citrus trees invest large amounts of carbohydrate resources in flowering. The high demand for carbohydrates by the flowers during anthesis may exceed the daily carbohydrate production by photosynthesis. In such cases, the tree must rely on carbohydrate reserves from other parts of the tree. The resultant excess demand for photoassimilates may temporarily upset the carbohydrate balance within the tree (Bustan and Goldschmidt, 1998).

Hormones and growth regulators

The precise role of hormones and the interactions among phytohormones and with other factors (e.g., carbohydrates) on the control of citrus flowering is still relatively unknown. Most of the available information has been derived from exogenous application of hormones and growth regulators. Research on hormone endogenous levels and their various interactions has been limited. The most important hormones for which a role on citrus flowering has been determined include gibberellins, cytokinins, auxins, ethylene, and abscisic acid.

Gibberellins. The effects of gibberellins on citrus flowering have been inferred by association of relative high or low levels of exogenous and endogenous gibberellins with their corresponding flowering response and not by direct cause-effect evidence (Koshita et al., 1999).

Gibberellins constitute the only phytohormones that consistently inhibit citrus flowering if applied before the beginning of differentiation. Under subtropical conditions, if gibberellic acid (GA₃) is applied during the winter rest period, it inhibits flower bud induction completely (Monselise and Halevy, 1964; Spiegel-Roy and

Goldschmidt, 1996). Applications of GA₃ to water stressed trees also prevented flower formation. These applications reportedly became inactive when applied after 6 days into the differentiation period (Nir et al., 1972).

Gibberellins can significantly reduce the number of buds that have been induced to flower (Monselise and Halevy, 1964). Although applications of GA₃ sprays have been reported to inhibit generative shoot production while vegetative shoots remain the same (Goldschmidt and Monselise, 1972), there are also reports that GA₃ increases vegetative shoot production (Lord and Eckard, 1985).

Studies on endogenous gibberellins also indicate that high levels reduce flower bud formation. Gibberellin content in the leaves is significantly higher in bearing shoots than in vegetative shoots. Flowering buds are fewer in the bearing shoots than in nonbearing shoots (Koshita et al., 1999). Gibberellin activity is also higher in fruiting shoots late in the on-year than in nonfruiting shoots late in the off year (Saidha et al., 1983).

Cytokinins. High cytokinin levels have a stimulating role on citrus flower bud differentiation. Cytokinins stimulate cell division and lateral bud initiation. Cytokinins also cause the flowering buds to function better as sinks for translocated metabolites (Saidha et al., 1983). Cytokinins are used during periods of active shoot growth, particularly when rapid leaf-expansion is taking place (Hendry et al., 1982). In studies of flower bud development, in vitro cytokinins induced the sprouting of adventitious buds.

Changes in cytokinin levels throughout floral development have a possible role in the enhancement of flower bud differentiation. An increase in the endogenous levels of cytokinins starts at an early stage of bud differentiation. Cytokinin contents in the floral

buds increase rapidly when the morphological differentiation period begins and levels off in the formation stage of the sepals (Lifeng et al., 1992). Maximum cytokinin levels occur in ovaries of most cultivars at anthesis, and fall sharply later during the rapid growth phase. Levels appear relatively higher in flowers from the "leafy" inflorescences than in those from the leafless type. Higher cytokinin levels are associated with fruit set (Hernández-Miñana and Primo-Millo, 1991; Saidha et al., 1985).

Cytokinins are synthesized in roots and are translocated through the xylem to other plant organs (Saidha et al., 1983). Adventitious roots of rooted cuttings, which are known to produce cytokinins, increased the number of inflorescences formed and increased the ratio of reproductive to vegetative shoot types (Oslund and Davenport, 1987). This indirect evidence, along with increased endogenous cytokinin level at initiation, suggests that cytokinins may promote of flower development. Cytokinins may enhance the number of flowers produced through increased flower formation and by enhancing the sprouting of reproductive shoots (Lifeng et al., 1992; Oslund and Davenport, 1987).

Abscisic acid. Based on the natural production of abscisic acid (ABA) during flower development, and the effects of exogenous applications of ABA on flowering response, a number of roles have been proposed for ABA. The increase in ABA levels in December and February has been associated with an increase of leafless inflorescences. Therefore, it is believed that ABA has a role on flower bud development (Koshita et al., 1999). The concentration of ABA reached a maximum 7 days after flower opening, thus suggesting a role in the enhancement of floral abscission. The effect of exogenous ABA reportedly changed during the season. Exogenous application of ABA to citrus buds at

different stages reduced bud sprouting, increased floral buds, or decreased the number of vegetative shoots (Okuda et al., 1996).

Auxins. Auxins are generally not implicated in citrus flowering. They are believed to have a negative effect on flowering based on the dominance of the apical flower, and the fact that bending of twigs increases flowering in lemon (Spiegel-Roy and Goldschmidt, 1996). Indirect roles may be related to possible flower inhibition, or to changes in sprouting patterns when applied externally (Guardiola et al., 1977).

Ethylene. Ethylene is produced naturally throughout floral development. However, in trials with exogenously applied ethylene, no stimulation of flowering was found (Moss, 1970). Although ethylene is produced at different stages of flower development, and by different floral organs, its role has not been completely determined. Young flower buds produce high levels of ethylene, possibly contributing to high abscission rates at that stage of development. At anthesis, the progressive curvature of citrus petals is associated with substantial increases in the production of ethylene. In fact, the major source of ethylene evolved is the petals, where rates are fivefold higher than in other flower organs. This increase in ethylene production may be related to petal abscission (Zacarias et al., 1991). Another peak of ethylene production occurs at fruit set, shortly after petal fall, suggesting a role in abscission at that stage of development (Bustan and Goldschmidt, 1998).

Plant nutrition

The spring flush and flower bud differentiation bring about the greatest number of changes in the allocation of nutritional resources. Nitrogen, phosphorus, and other mineral content levels are of particular importance for the flowering process.

During spring flush, mineral nutrient uptake by the roots is low, and the growth of the developing organs strongly depends on the re-translocation of reserves. Phosphorus and potassium are translocated more readily than nitrogen, and the amount of those elements that are retranslocated from old leaves may represent up to 70% of the total amount used in the formation of new organs until flower opening (Sanz et al., 1987).

Nitrogen. The nitrogen ($\text{NH}_3 - \text{NH}_4^+$) status of the buds has been related to the number of floral shoots and total number of flowers initiated. In addition, during the spring flush, high N levels may induce excess vigor and produce increased vegetative growth (Lovatt et al., 1988a). During floral induction, stress influences the accumulation of ammonia or ammonia-based compounds in aerial organs of the tree (Lovatt et al., 1988b). During flower development, a number of changes occur in citrus leaves. Concentrations of N (in the form of polyamines and other N based compounds) and dry weight increase as flowers develop (Cameron and Appleman, 1934; Kushad et al., 1990). More than 70% of N supplied to the new organs (flowers) during spring comes from N stored in old leaves, one-year-old-spring flush, twigs, and roots. Old leaves are the main reserve organs, contributing 40-50% of the total N exported to new bud growth. Roots and aerial woody tissues export between 30-35% and 15-25% of total reserve N, respectively (Legaz et al., 1995). Less than 3% of the absorbed N reaches ovaries and young fruits. During flowering, N content increases in petals, spring flush leaves, and ovaries. After petal fall, N is concentrated in young fruit, new flush leaves, and new flush twigs (Legaz et al., 1982).

Manipulation of foliar N content (by means of applying foliar urea) enhances floral intensity by improving the conditions that convert vegetative to reproductive buds.

N based compounds (polyamines) are reported to increase during inductive temperatures and after application of foliar urea (Ali and Lovatt, 1995). The effect of urea may be through the rapid release of ammonia in the plant tissue (Albrigo, 1998). Increased N levels serve as substrate to initiate the flowering process (Lovatt et al., 1988a).

Phosphorus. Values of phosphoric organic compounds (acid soluble phosphorus, acid insoluble phosphorus, phospholipids, phosphoproteins, nucleotids of RNA and DNA) increase as the spring flush of growth develops. Maximum levels are obtained during full bloom. Subsequently, these values decrease again (Parra and Ortuño, 1981). During the induction period, PO_3 applications can enhance flower bud induction possibly as the effect of increased stress (Albrigo, 1998).

Other minerals. Mineral elements in old leaves decrease during the spring flush of growth and reach minimum values at flower opening, coinciding with a peak during floral abscission. This is followed by a rapid recovery by the leaves of P and K with little change afterwards (Sanz et al., 1987).

Bud and shoot age

Bud age and position along the shoot affect flowering and bud sprouting. Younger buds flower more readily than older buds (Guardiola, 1981). Although some flowers arise on older branches, only buds younger than one year old contribute significantly to the formation of spring growth (Guardiola et al., 1977; Lovatt et al., 1984). The fraction of buds sprouting in spring decreases with increasing bud age. Summer buds contribute with a greater fraction of sprouting buds than older buds (previous spring or older). Vegetative buds sprout more readily than reproductive buds on shoots of the previous summer shoots, but the same could not be demonstrated in spring (older) shoots (García-Luis et al., 1995b). Bud sprouting requirements are

different for buds of different ages. Buds from previous spring scarcely sprout without being exposed to low temperatures. Therefore, spring buds require a longer chilling period than summer buds for sprouting (García-Luis et al., 1995b).

Because few reports disclose position within the canopy of different aged stems, it is not possible to determine whether heaviest flowering is associated with terminal position of certain aged stems or with bud age *per se* (Krajewski and Rabe, 1995). Differences in sprouting and flowering may be due just to age differences of the buds. However, the position within the canopy of summer flushes may affect sprouting and flowering. This is because summer flushes are predominantly on the outside of the canopy. As stems fall under progressively stronger apical control, summer flushes are more prone to sprouting. Furthermore, older wood also becomes shaded, which tends to inhibit bud break (Lewis and McCarty, 1973).

Crop load

The presence of crop load (or effects of recently removed fruit) located at the apex of branches inhibits the production of new shoots and the expression of vegetative and any floral nature of those shoots in basipetal lateral buds (Moss, 1969; Southwick and Davenport, 1987). Fruits do not seem to exert a strong inhibitory effect on the vegetative and floral growth of other nearby tree branches (Southwick and Davenport, 1987). The effects of fruit on spring growth are threefold: 1) fruit presence diminishes significantly the proportion of buds that are able to sprout without being chilled (García-Luis et al., 1995b); 2) fruit load also has an inhibitory effect on the production of vegetative flushes at bud sprouting in spring (Goldschmidt and Monselise, 1972; Reuther, 1973); and 3) the development of floral buds is diminished by the presence of the fruit (Koshita et al., 1999; Moss, 1969).

Crop load effects on citrus flowering may be related to carbohydrate and/or hormonal interactions. Fruit load may act as a strong sink for carbohydrates, reducing the flowering potential. Gibberellin content is significantly higher in the leaves of fruit bearing shoots than in those of vegetative shoots (Koshita et al., 1999). Gibberellins and carbohydrates operate as independent regulatory factors in the flowering of citrus (Erner, 1988).

The enhancement of flowering caused by early fruit removal may be related to increases in carbohydrate availability, changes in hormonal levels, or a combination of both. While there is evidence that crop load enhances the photosynthetic rates of bearing trees (Lenz, 1978), early fruit removal resulted in an increase in carbohydrate levels by means of enhanced photosynthesis, and the removal of carbohydrate sinks (García-Luis et al., 1995a; Okuda et al., 1996). Fruit removal also caused changes in hormonal levels such as reduced gibberellins and ABA levels in the leaves (Goldschmidt et al., 1985; Moss, 1971; Okuda et al., 1996). The reason for increased flowering upon fruit removal is inconclusive as removing the fruits involves eliminating an important carbohydrate sink, but at the same time upsets the hormonal balance by eliminating a major natural source of gibberellins (Goldschmidt et al., 1985; Moss, 1971).

In Vitro Flowering

Early attempts to produce flowers in vitro resulted in the production of vegetative shoots exclusively (Altman and Goren, 1974; Guardiola et al., 1982). The first successful report on achieving the production of flowers in vitro was performed on induced buds cultured in a medium supplemented with sucrose and a cytokinin (García-Luis et al., 1989). Further studies failed to induce flowers by exogenous application of hormones,

growth regulators, and high concentrations of sugars. However, buds were successfully induced to flower in vitro when exposed to low temperature inductive conditions (Tisserat et al., 1990).

Performance of buds in vitro does not necessarily reflect the behavior of buds in vivo. In the first study, the percentage of flowering explants was always lower than the percentage of naturally flowering buds in spring (García-Luis et al., 1989). Although citrus buds cultured in vitro seem to have higher low-temperature requirements for the induction of citrus flowers than intact buds (Tisserat et al., 1990), the effect of low temperature on the enhancement of bud sprouting is comparable. However, treatments such as ringing which increase flowering in vivo also increased the number of explants flowering in vitro (García-Luis et al., 1989). Similarly crop load, which reduces generative growth in vivo, also reduced reproductive growth in vitro (García-Luis and Kanduser, 1995).

Phenology Studies of Citrus Flowering

Modeling of Agricultural Systems

Agricultural models include mathematical equations that are used to define a physical system (Jame and Cutforth, 1996). Models constitute convenient ways of synthesizing complex systems into single packages. Therefore, agricultural modeling has become a useful tool in integrated studies of crop-weather relationships (Ben Mechlia and Carroll, 1989b).

Objectives of phenological studies and models include the observation of developmental events, accurate explanation of historical information, and predictive ability (Frometa et al., 1979; Schwartz et al., 1997; Ben Mechlia and Carroll, 1989a; Ben

Mechlia and Carroll, 1989b). Crop models are quantitative schemes for the prediction of growth, stage of development, and yield of a given crop (Montieth, 1996). Models are being developed to inform growers of the stage of development of their crop during the growing season, which can be used to efficiently schedule farm operations (Schwartz et al., 1997). Crop models may also be used as a tool in teaching, research, or management decision-making. Decisions can be made for profitable crop production, for work under different environmental conditions, and for cultivar selections (annual crops, primarily) (Jame and Cutforth, 1996).

Crop models must overcome user distrust of computer-generated information. One way to gain user's confidence is to demonstrate the predicting power of the crop model (Hayes and Privette III, 1998). In agriculture, long-term success of crop models depends on their ability to supply farmers with useful information in an economical and easily accessible form (Jones, 1990). As growers become increasingly receptive to computer use (Ferguson and Israel, 1998), this provides an opportunity to provide them with useful models for their decision-making processes.

Expert Systems and Decision Support Systems

Artificial intelligence refers to the capability of a device to perform functions that are normally associated with human intelligence, such as reasoning and optimization through experience. Expert Systems (ES) and Decision Support Systems (DSS) are artificial intelligence tools that mimic human reasoning. An ES is a computer program aimed at mimicking the human expert's reasoning process and knowledge in specific fields (Bentham, 1998; Jackson, 1990; Waterman, 1986). In agriculture, most ES manipulate knowledge, search for connections and relations among different patterns, and provide diagnostics or recommendations to nonspecialists (Nolasco-Bethencour, 1998).

A Decision Support System (DSS), in comparison to an ES, is a computer program that solves problems spanning over several knowledge or problem areas. This is accomplished by combining information, knowledge, and human expertise; through integrating of expert systems, rule-sets, site-specific data, and any other combination of software and/or information that can aid in the decision-making process through interactions with the end-user (Bentham, 1998).

In citrus management, production practices usually require specific tree phenological status for maximum efficiency. Therefore it was considered that for citrus, an ideal DSS should have a phenology-based component to match production practices to the actual needs of field trees. Considering fruit production, each season begins with the induction and development of floral buds. A number of temperature-based phenological models have been developed to explain and predict citrus flowering (Bellows and Morse, 1986; Ben Mechlia and Carroll, 1989b; Lovatt et al., 1984). All these models are statistical relations based on local weather factors. These models were developed for Mediterranean type climates, which make them generally unsuited for other production regions (i.e., tropical or subtropical humid regions).

Modeling Phenological Processes

Phenology is the response of organisms to changes in their environment. The primary objective of phenology studies was traditionally to observe the occurrence (dates of start and termination) of phenological events (Frometa et al., 1979). The current phenological studies include the development of models able to explain the occurrence of phenological events (Schwartz et al., 1997). All phenological models are simplifications of real-living processes that should explain historical information accurately and have good predictive ability (Ben Mechlia and Carroll, 1989a; Ben Mechlia and Carroll,

1989b). In fact, the ability to predict the performance of a given cultivar in a particular situation has been a major goal of agricultural modeling. Other desirable characteristics for phenological models include universality, ease of implementation, and the use of data readily available (Ben Mechlia and Carroll, 1989a; Ben Mechlia and Carroll, 1989b).

Modeling Citrus Flowering

The most important exogenous factors affecting citrus flowering include temperature (air and soil temperature), and soil-water relations. While citrus trees growing in tropical areas induce flower buds primarily by the effects of drought stress (Cassin et al., 1969; Ginestar and Castel, 1996; Southwick and Davenport, 1986), ambient or air temperature is the most important exogenous factor affecting floral induction and development in subtropical environments (Cassin et al., 1969). A number of phenological models have been developed to explain and predict citrus flowering under subtropical environments (Bellows and Morse, 1986; Ben Mechlia and Carroll, 1989b; Lovatt et al., 1984). All these models are temperature-based and were developed using weather data of the local region.

Lovatt et al. (1984) developed a temperature driven model to predict the phenology of citrus flowering (date of occurrence). This model provides the degree-day requirements needed to go from a specific calendar date to anthesis or petal fall. These degree-day requirements are based on statistical procedures that minimized the cumulative mathematical variability along the years. Thus, the onset of differentiation and the degree-day threshold is based on a calendar date and a temperature in which the error was minimized. This model may be used to explain the historic phenological observations, but is not useful for predictive purposes or in other citrus locations, as

climatic conditions vary from year to year and from location to location. A single calendar date as a starting date is therefore not a logical, explainable point of reference.

Other citrus flowering models include those from Bellows and Morse (1986) and Ben Mechlia and Carroll (1989). Both of these models provide a set of mathematical functions relating phenological parameters to local weather data (temperature primarily). These models consist of statistical relations based on local weather factors. These models may be considered oversimplifications of citrus flowering processes, and fail to account for other factors influencing the flowering process. In particular, these models fail to account for the two phenological processes involved: induction and differentiation. Furthermore, as these models were developed for a Mediterranean type climate, they may not permit general application over a wide geographical area (i.e., subtropical humid or tropical regions).

All the models to explain and predict citrus flowering developed so far consist of statistical relations based on local weather factors. Statistical models tend to emphasize the climatic features of the area for which they were developed. Although statistical models may have good fit, they also have little universality. An ideal model should use physiological parameters that emphasize physiological response to environmental conditions.

This study presents a different approach in the modeling of citrus flowering that uses phenological parameters for monitoring of flower bud induction and prediction of date to peak anthesis once initiation of differentiation has been determined. In addition, this study implemented expert system rules for the management of other factors for

which information is scarce such as date of initiation for single or multiple blooms, and cultivars.

CHAPTER 2
EFFECT OF LOW TEMPERATURE STRESS LEVELS, CROP LOAD, AND BUD
AGE ON THE FLORAL INDUCTION OF SWEET ORANGE TREES [*Citrus sinensis*
(L.) Osbeck] IN CENTRAL FLORIDA

The flowering process in citrus consists of flower bud induction, differentiation, and expansion processes preceding anthesis (flower opening). Both phenological processes (induction and differentiation) leading to bloom are primarily temperature dependent in subtropical climates and water-stress dependent in tropical climates (Cassin et al., 1969). Induction refers to the triggering of the flowering process by environmental factors resulting in a subsequent change in the pattern of bud development, leading to flowering (Guardiola, 1997). Under subtropical conditions, vegetative buds are induced to be floral buds by low temperatures (Reuther et al., 1973). Under tropical conditions flowers are induced during the drought stress period, but the beginning of reproductive growth does not occur until the onset of the rain period (Cassin et al., 1969). As in flower bud induction caused by low temperatures, the duration, intensity, and time course of the drought stress directly influence timing and intensity of citrus flowering (Furr and Armstrong, 1956; Ginestar and Castel, 1996).

Differentiation involves the anatomical and morphological transition of a vegetative meristem to a floral meristem (Davenport, 1990). Vegetative and reproductive meristems are anatomically indistinguishable before differentiation starts, even if the flowering meristem is fully induced. In citrus, the differentiation of floral organs does not occur until the first stages of bud swelling and sprouting. The time of initiation of differentiation varies slightly by citrus cultivar (Abbott, 1935). The morphological

development of the flowers immediately follows the initiation of the flower primordia without interruption. The rate of development depends on temperature and the number of flowers differentiating (Guardiola, 1997; Moss, 1973). Anthesis (flowering) occurs after the induction and differentiation processes have been completed, usually when warmer temperature conditions exist (Bellows and Morse, 1986; Lovatt et al., 1984).

Flower Bud Induction

Induction is the mechanism that results in the commitment of meristematic cells to the formation of reproductive structures. Induction is an activating or de-repressing mechanism of the flowering process brought about by environmental factors. The predominant flower bud induction stimulus varies according to the environmental conditions to which the trees are exposed. In fact, flowering in citrus is considered day-neutral and self-inductive with no single, indispensable induction stimulus known (Moss, 1969). Climatic conditions play a significant role on the vegetative and reproductive phases of citrus. Citrus trees growing in tropical conditions flower throughout the year with peak levels occurring when rainfall follows periods of dry weather. Under these conditions, flowers are induced during the drought stress period, but the beginning of reproductive growth does not occur until the onset of the rain period (Cassin et al., 1969). Under subtropical conditions, citrus trees produce flowers primarily during the spring following the winter season (García-Luis et al., 1995a; Simanton, 1969). Induction occurs during the winter-rest period as inhibitors applied at this time inhibit flower formation (Monselise and Halevy, 1964). The first step towards the induction of flowers is a cessation of vegetative growth caused by low temperatures. Low temperatures also cause the buds to remain inactive during the winter season, while they are induced to

flower (Reuther et al., 1973). The release of the stress by higher temperatures signals the beginning of reproductive growth (Cassin et al., 1969).

Regardless of the similarity in flowering response, flower bud induction in both situations is not mediated through a common decrease in xylem pressure potential (Southwick and Davenport, 1986). Although no conclusive explanation has been provided for the similarity in flowering response, the possible role of a root signal to cause bud break and flowering has been proposed (Poerwanto and Inoue, 1990).

Monitoring Flower Bud Induction

The duration, intensity, and time course of the stress influence timing and intensity of citrus flowering (Furr and Armstrong, 1956). The primary technique available for induction monitoring of field citrus trees is the girdling and defoliation method. This method is based on the concept that leaves perceive the low temperature induction stimulus, and translocate the induction stimulus through the phloem. Therefore, after girdling and defoliation, no inducing stimulus can reach the buds above the girdle. The girdling and defoliation method also assumes that girdling does not affect the percentage of sprouting buds (Ayalon and Monselise, 1960; Furr and Armstrong, 1956; Furr et al., 1947)

Since the early studies on girdling and defoliation, a number of drawbacks to the girdling and defoliation technique have been identified. Although the presence of leaves does have a direct role during the induction period (defoliating before low temperature stress diminishes the flowering response), *in vitro* studies of inductive conditions suggested that the induction stimulus could also be perceived at the bud itself (García-Luis et al., 1992; Tisserat et al., 1990). Girdling (without defoliation) tends to increase flowering by changes in carbohydrate levels distal to the girdle and its effects on

hormonal levels (Erner, 1988; Wallerstein et al., 1973). The effect of girdling is limited to phloem transportable factors only (Altman and Goren, 1974). Despite the common use of the girdling and defoliation technique, no study has analyzed the hormonal and/or carbohydrate changes following girdling and defoliation.

In the absence of a better technique, and regardless of these drawbacks, the girdling and defoliation technique continues to be accepted as the best technique to monitor induction throughout the winter period of field citrus trees.

Effect of Ambient Temperatures

Under subtropical environments, ambient or air temperature not only has a direct effect on induction and differentiation but also affects flowering date, morphological characteristics, and flowering intensity (Cassin et al., 1969; García-Luis et al., 1992). Flowering date depends on the temperature of the inductive winter season. During regular winter seasons, a cold winter will produce a flowering period later than the average (Lomas and Burd, 1983). However, high day temperatures during floral organ differentiation reduce flower development time and therefore advance date of anthesis (Moss, 1973). Ambient temperatures also influence morphological characteristics of flowers and ovaries. High ambient temperatures are associated with smaller flowers and with small and long ovaries (Inoue, 1989). At low air temperatures, flowers and ovaries are bigger than at higher temperatures. As for flower intensity, high temperatures during bud sprouting are reported to increase the leafiness of the inflorescences and to inhibit flowering. By contrast, number of flowers per tree, number of flowers per node, and sprouting rate are greater at low air temperatures (Poerwanto and Inoue, 1990).

Effect of Crop Load

The presence of crop load (or effects of recently removed fruit) located at the apex of branches inhibits the production of new shoots as well as expression of the vegetative and floral nature of those shoots in basipetal lateral buds (Moss, 1969; Southwick and Davenport, 1987). Fruits do not exert a strong inhibitory effect on the vegetative and floral growth of other tree branches (Southwick and Davenport, 1987). The effects of fruit on spring growth are three fold: 1) fruit presence diminishes significantly the proportion of buds that are able to sprout (García-Luis et al., 1995b); 2) fruit load also has an inhibitory effect on the production of vegetative flushes before bud sprouting in spring (Goldschmidt and Monselise, 1972; Reuther, 1973); and 3) the development of floral buds is diminished by the presence of the fruit (Koshita et al., 1999; Moss, 1969).

Crop load effects on citrus flowering may be related to carbohydrate and/or hormonal interactions. There is evidence that crop load enhances the photosynthetic rates of bearing trees (Lenz, 1978). However, fruit load may also act as a strong sink for carbohydrates reducing the flowering potential. Gibberellin content is significantly higher in the leaves of fruit bearing shoots than in those of vegetative shoots (Koshita et al., 1999). According to Erner (1988), gibberellins and carbohydrates operate as independent regulatory factors in the flowering of citrus.

The enhancement of flowering caused by fruit removal may be related to increases in carbohydrate availability, changes in hormonal levels, or a combination of both. Early fruit removal resulted in an increase in carbohydrate levels by means of enhanced photosynthesis and the removal of carbohydrate sinks (García-Luis et al., 1995a; Okuda et al., 1996). Fruit removal also caused changes in hormonal levels and

reduced gibberellins, and ABA levels in the leaves (Goldschmidt et al., 1985; Moss, 1971; Okuda et al., 1996). However, reports suggesting that flowering increases after fruit removal are inconclusive. Fruit removal eliminates an important carbohydrate sink but at the same time upsets the hormonal balance by eliminating a major natural source of gibberellins (Goldschmidt et al., 1985; Moss, 1971).

Effect of Bud Age

Bud age and its position along the shoot affect bud sprouting and flowering. The proportion of buds sprouting in spring decreases with increasing bud age. On summer shoots, vegetative buds sprout more readily than generative buds, but the same could not be demonstrated in spring (older) shoots (García-Luis et al., 1995b). Younger buds flower more readily than older buds (Guardiola, 1981). Although some flowers arise on older branches, only buds younger than one year old contribute significantly to the formation of spring growth (Guardiola et al., 1977; Lovatt et al., 1984). Because few reports disclose position within the canopy of different aged stems, it is not possible to determine whether heaviest flowering is associated with terminal position of stems or with bud age *per se* (Krajewski and Rabe, 1995). Differences in bud sprouting and flowering may be due just to age differences. However, the position within the canopy of these flushes may also affect bud sprouting and flowering. Stems fall under progressively stronger apical control as they lose the terminal positions within the canopy and give rise to new flushes. Older wood also becomes shaded, which tends to inhibit bud break (Lewis and McCarty, 1973). Low-temperature requirements for bud sprouting are different for trees carrying crop load. The spring flush almost never sprouts without low temperatures. Therefore, spring buds require longer low-temperature exposure than summer buds for bud sprouting (García-Luis et al., 1995b).

Low-temperature inductive conditions are directly related to bloom intensity by influencing the type of inflorescence being formed. The number of hours at low temperatures has a large effect on the type of growth arising from axillary buds formed: vegetative (with no flowers), mixed (with flowers and leaves), or generative (leafless with one to several flowers) (Moss, 1969). Although this general mechanism is widely accepted, there have been no efforts to quantify this response to low temperature inductive conditions. The objective of this study was to quantify the phenological response of citrus trees to low-temperature winter conditions and crop load.

Materials and Methods

Plant Materials

This study was conducted in two locations near Lake Alfred, Florida. The first location was a commercial grove containing mature, field grown 'Hamlin' sweet orange trees [*Citrus sinensis* (L.) Osbeck] grafted on Carrizo citrange [*Citrus sinensis* (L.) Osbeck \times *Poncirus trifoliata* (L.) Raf.] rootstock. In this location 14 and 15 healthy, uniform trees were selected for the first and second year of study, respectively. The second location consisted of 6-year-old 'Valencia' sweet orange trees [*Citrus sinensis* (L.) Osbeck] grafted on Swingle citrumelo [*Citrus paradisi* Macf. \times *Poncirus trifoliata* (L.) Raf.] rootstock. For this location a total of 14 and 16 trees were used for the first and second year of study, respectively. Both locations were under commercial horticultural practices for the region that included periodical irrigation throughout the winter of both seasons.

Experimental units consisted of pairs of branches holding two different levels of crop load. Selection of each pair of branches was done until the summer flush was

complete. Every pair of branches was selected from the same tree location (quadrant and height), with similar diameter base, but differing in crop load (with and without developing fruit). No fruit was removed to adjust for crop load. On all the branches a number of individual shoots were selected and tagged according to their age (spring or summer flushes of growth). Pairs of branches were numbered and mapped identifying their tree number and spatial position within each tree.

Pairs of branches were randomly assigned to each particular treatment minimizing the effect of individual trees or spatial locations of the branches on the phenological response. Each pair of branches was assigned to different treatments avoiding the repetition of treatments within the same tree, and the same spatial location in different trees, and minimizing the same combination of treatments in different trees.

Experimental Design

This study was conducted during the 1998-1999 and 1999-2000 seasons (hereinafter called the 1999 and 2000 seasons, respectively). Completely randomized designs with different induction treatments and two crop load treatments were setup for 'Hamlin' and 'Valencia' trees (Table 2-1). A total of 8 and 7 induction treatments were administered to the 'Hamlin' trees for the 1999 and the 2000 seasons, respectively. 'Valencia' trees were exposed to a total of 7 and 8 induction treatments for the 1999 and 2000 seasons, respectively. Number of treatments depended on whether or not buds had sprouted at the moment in which the interruption of induction was scheduled for the set of branches. Induction treatments consisted of different induction levels before the interruption of induction. Different induction levels were administered to both cultivars by exposing the branches to low-temperature inductive conditions and by periodically interrupting the induction process. The girdling and defoliation method was used to

interrupt the induction process for each pair of branches (Furr et al., 1947). Crop load treatments refer to the presence or absence of fruit on each individual branch. In both locations, each treatment was replicated six times. Each replication consisted of different pair of branches.

Starting 1 Nov, induction was interrupted twice monthly on different sets of pairs of branches each time. For the 1999 season, induction was interrupted on 3 and 17 Nov, 4 and 18 Dec, 4 and 19 Jan, and 2 and 16 Feb. For the 2000 season induction was interrupted on 3 and 18 Nov, 3 and 16 Dec, 3 and 18 Jan, and 4 Feb. Each induction treatment was identified by the consecutive fall-winter date in which induction was interrupted on each pair of branches. For both years and cultivars, one set of branches was left to complete their normal flowering cycle. No manipulations of any kind were performed on these branches. These branches were used as control branches. In addition, the control branches were used to determine seasonal and crop load variations.

Data Collection

Data were collected until there was certainty of the final number of flowers on the branches. This generally occurred after the floral buds were sufficiently developed to provide an accurate count (from the "pop-corn stage" on). Collection of data continued through anthesis. Along with the phenological data, the date of data collection for each individual branch was recorded. Data collection focused on branch characteristics, as well as on bud sprouting and flowering data.

Branch characteristics. Data on the characteristics of the branch included cultivar, branch identifier, induction and crop load treatments, and number of shoots. Induction treatments were identified by the date in which induction was interrupted on each pair of branches. Crop load treatments refer to the presence or absence of fruit on the branch.

Number of shoots is the total number of individual shoots able to bear flowers. This parameter was needed to estimate the size of the branch for the homogenization of flowering and bud sprouting parameters.

Bud sprouting data. Collected bud sprouting data included the total number of vegetative and reproductive shoots, and combined the total number of sprouting buds. A characterization of the sprouting buds was done by first determining the nature of the flushes of growth (vegetative or reproductive). The total number of vegetative buds was obtained by counting all the new growth in which no reproductive structures (flowers) could be observed. As each reproductive bud was recorded and counted, it was possible to obtain the total number of buds with reproductive growth. Total number of sprouting buds included both reproductive and vegetative bud growth.

Flowering data. Collected flowering data included total number of buds with flowers, total number of flowers per branch, total number of flowers in the inflorescence, and the position of the sprouting bud along the shoot. The bud in the apex-most position was considered to be in Position 1, the bud in the following position was considered in Position 2, and so on. Flowering data obtained from the shoots that had been previously marked as spring or summer shoots were collected as described above, specifying the age of the shoot from which the data came.

Phenological parameters (bud sprouting and flowering) were developed due to the difference in size of the experimental units. Bud sprouting parameters included the total numbers of sprouting, reproductive, and vegetative buds divided by the total number of shoots on each branch. Flowering parameters included flowers-per-shoot, flowers per flowering bud, and flowers per sprouting bud. The flowers-per-shoot parameter related

the total number of flowers divided by the total number of shoots able to bear flowers. The flowers-per-flowering bud parameter related the total number of flowers by the total number of buds that sprouted with reproductive growth (average number of flowers per inflorescence). Total number of flowers per sprouting bud was obtained by dividing the total number of flowers by the total number of sprouting buds.

Analysis 1. Phenological Response (Bud Sprouting and Flowering Parameters) of 'Valencia' and 'Hamlin' Trees as Affected by Season and Crop

Effect of season and crop load on bud sprouting parameters

Characterization of the phenological response of 'Valencia' and 'Hamlin' trees was done using the set of branches to which no manipulations was performed and that was left to complete their normal flowering cycle (control branches). The effect of seasonal differences (from the 1999 to the 2000 season) on the phenological response of 'Valencia' and 'Hamlin' trees was evaluated in terms of differences in phenological parameters such as bud sprouting and flowering parameters.

For each cultivar, differences in the phenological parameters for bud sprouting were analyzed comparing the mean value for each year by crop load. As sprouting buds may develop either vegetative or reproductive organs, t-tests were carried out on the effect of the flowering season on the total number of sprouting buds per shoot by crop load, as well as on the total number of buds with vegetative and reproductive growth per shoot by crop load. In addition, bud-sprouting parameters were also subjected to t-tests comparing the mean value for each crop load by year. All t-tests were done using PROC TTEST (SAS, NC State Version 7). Significance values were set at $P \leq 0.05$ for significant (*), and $P \leq 0.01$ for highly significant (**). Values above $P > 0.05$ were considered as non-significant (NS).

Effect of season and crop load on flowering phenology

Since the collection of flowering data was done until there was certainty in the numbers of flowers being formed ("pop-corn" stage to anthesis), dates of data collection of the control branches provided a good estimation of the length of the flowering period for both seasons.

Similarly for each cultivar, flowering parameters (flowers-per-shoot, flowers-per-flowering bud, and flowers-per-sprouting-bud) were subjected to t-tests comparing the mean value for each year by crop load. t-tests were also run for the flowering parameters comparing the mean value for each crop load by year.

Floral distribution by shoot age (summer vs. spring)

Floral distribution by shoot age (summer vs. spring) was performed using the flowering data obtained from the shoots that had been previously tagged as spring or summer flush for 'Hamlin' and 'Valencia' cultivars. Data collected from each individual shoot included total number of flowers and their distribution according to their position along the shoot. As there were equal numbers of marked shoots from each corresponding age, it was possible to estimate percentages of flowers coming from spring or summer shoots, as well as their positional distribution along the shoot. This information was in turn used for the determination of differences in induction requirements by shoot age.

Analysis 2. Effect of Different Induction Levels on the Phenological Response of 'Valencia' and 'Hamlin' Trees

Effects of different induction and crop load treatments on phenological parameters

The effect of different induction levels on the phenological response of 'Valencia' and 'Hamlin' trees was done during the 1999 and 2000 seasons. Differences in the magnitude of the phenological parameters between the two cultivars prevented the

combined use of phenological parameters of both cultivars. Furthermore, although the interruption of induction was performed on the same calendar dates of the 1999 and the 2000 seasons, it was not possible to consider them as true repetitions due to differences in seasonal inductive conditions. Therefore, the analyses of variance (ANOVAS) statistical analyses were performed separating between years and cultivars.

The effects of the induction (girdling and defoliation) and crop load treatments on phenological parameters (sprouting and flowering) were tested with ANOVAS. If the effects were determined significant at the 5% value, further analyses were performed. 'Duncan' analyses were performed to separate phenological differences considering induction treatments as discrete factors.

Effects of different induction levels on floral distribution by bud position along the shoot

The effects of different induction levels and crop load treatments on the floral distribution by bud position along the shoot were determined using analyses of variance. ANOVAS were run using the total number of flowers of shoots on each position divided by the total number of shoots able to bear flowers. Significance values for the analyses of variance were set at $P \leq 0.05$ for significant (*), and $P \leq 0.01$ for highly significant (**). Values above $P > 0.05$ were considered as Non-Significant (NS).

Analysis 3. Effect of Weather Data on Phenological Response of 'Valencia' and 'Hamlin' Trees

Induction as a function of weather

Under subtropical conditions, the duration, intensity, and time course of the low temperature stress influence timing and intensity of citrus flowering (Furr and Armstrong, 1956). The objective of this study was to relate the phenological response to local weather information and crop load.

Weather data

Real-time weather measurements were obtained from the Florida Agriculture Weather Network (FAWN) system. The FAWN system is an online weather information system that provides real-time weather data for a variety of locations throughout the state of Florida. Weather information from October to March of both seasons was obtained for the nearby Lake Alfred Station (Fig. 2-1). Temperature records were stratified into five-degree ranges (below 0, 0 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30 and above 30°C) and the number of hours within each temperature range was obtained. A cumulative value of hours within each temperature range was obtained for each treatment date.

Phenological response

Phenological parameters were related to local weather data using stepwise multiple regression analysis (SMRA) procedures (SAS, NC State Version 7). Phenological parameters (flowers-buds-per-shoot, flowers-per-shoot, flowers-per-flowering-bud, and vegetative-buds-per-shoot) were used as dependent variables and the cumulative number of hours at each temperature range and crop load status were used as independent variables.

Results

Analysis 1. Phenological Response (Bud Sprouting and Flowering Parameters) of 'Valencia' and 'Hamlin' Trees as Affected by Season and Crop

Effect of season and crop load on bud sprouting

Differences in bud sprouting parameters were determined using the set of branches that was left to complete its normal flowering cycle (control branches). In the absence of developing fruit, a significant increase from the 1999 to the 2000 season(s) on

the total sprouting buds per shoot was determined for both cultivars (Tables 2-2 and 2-3). This increase in total sprouting can be attributed to increases in the sprouting of flowering buds per shoot. Seasonal differences on the sprouting of vegetative buds were not found. By contrast, the sprouting of vegetative buds was significantly increased by the presence of fruit only for 'Hamlin' during the 2000 season.

The effect of crop load on the sprouting of the control branches was inconsistent between years and cultivars. Although, total bud sprouting was apparently not significantly affected by the presence of crop load in any of the two cultivars or seasons (Tables 2-2 and 2-3), the number of vegetative buds per shoot was significantly increased by the presence of crop load for 'Hamlin' during the 2000 season. The number of flowering buds per shoot was significantly reduced for 'Hamlin' during the 2000 season and for 'Valencia' during the 1999 season.

Effect of season and crop load on flowering phenology

The flowering period for the 1999 season spread over a much longer period of time than in the 2000 season. In 1999, data collection for both cultivars started on 25 Feb 1999 and continued until 3 Apr 1999 for a total of 37 d. In comparison, data collection for the 2000 season started on 8 Mar and continued on to 17 Mar 2001 for a total of 9 d.

Seasonal effects on the total number of flowers per shoot were inconsistent between cultivars. The effect of seasons was statistically significant for 'Hamlin' branches in the absence of fruit, and in 'Valencia' branches when fruit was present (Tables 2-4 and 2-5). Seasonal differences in flowers per flowering bud were not statistically significant in any cultivar or crop load. Inconsistencies were also found in flowers per sprouting bud. While this parameter was not significantly different from 1999 to the 2000 season for 'Hamlin' at both crop loads, the number of flowers per

sprouting bud was significantly increased during the 2000 when compared to the 1999 season for 'Valencia' branches at both crop loads.

There was also no consistent effect for crop load on all flowering phenological parameters across cultivars and years. For 'Hamlin' branches, crop load effects on flowering parameters were significantly reduced only in the number of flowers per sprouting bud for the 2000 season (Table 2-4). Differences in other 'Hamlin' flowering parameters (i.e., flowers per shoot and flowers per flowering bud) and seasons were not significantly affected by crop load. In 'Valencia', the presence of crop load caused statistically significant reductions in flowers per shoot, and flowers per sprouting bud for the 1999 season, and in flowers per flowering bud during the 2000 season (Table 2-5).

Floral distribution by shoot age (summer vs. spring)

For the same number of summer and spring shoots, summer shoots developed more flowers for both cultivars and years. For 'Hamlin' branches, summer shoots developed 69.7% and 72.6% of the total number of flowers per shoot for the 1999 and 2000 season, respectively. Combining the 1999 and 2000 seasons, summer shoots of 'Hamlin' trees developed 71.56% of flowers per shoot, whereas spring shoots developed 28.44% (Fig. 2-2). Similarly, in 'Valencia' branches, summer shoots contributed with 81.6% and 77.1% of the flowers per shoot for the 1999 and 2000 cycle, respectively. When combining the 1999 and 2000 seasons, summer shoots of 'Valencia' trees provided 78.21% of the total flowers, while spring shoots developed 21.79%.

The distribution of flowers per position along the shoot (for summer and spring shoots) for both cultivars showed a marked apical tendency (Table 2-6). 'Hamlin' buds in Positions 1 through 3 accounted for 64.2% and 80% of the total flowers for summer

and spring shoots, respectively. 'Valencia' buds in the same positions accounted from 74.3% and 84.5% of the total flowers of the corresponding shoot age.

Analysis 2. Effect of Different Induction Levels on the Phenological Response of 'Valencia' and 'Hamlin' Trees

Effects of different induction levels and crop load on phenological parameters

Different induction levels had a significant effect on all bud sprouting parameters (sprouting, vegetative, and reproductive buds per shoot) of 'Hamlin' trees during the 1999 and 2000 seasons (Table 2-7). For 'Hamlin,' the number of vegetative buds per shoot diminished as the winter progressed (increased induction levels) (Tables 2-8 to 2-11). In contrast, for 'Valencia' branches different induction levels did not significantly affect total bud sprouting, or the sprouting of vegetative buds during 1999. Greater induction levels only decreased the sprouting of vegetative buds during 2000. The sprouting of reproductive buds was consistently increased as the winter progressed and more inductive conditions developed. In both cases it was possible to establish more statistically equivalent groups for the sprouting of reproductive buds than those with vegetative growth.

The presence of crop load significantly increased the sprouting of vegetative buds, and decreased the sprouting of reproductive buds for 'Hamlin' during both years. By contrast, the effects of crop load only reduced the sprouting of reproductive buds during the 2000 season for 'Valencia.'

The total numbers of flowers per shoot, and per sprouting bud were significantly increased as the winter progressed for both cultivars and seasons (except for flowers per flowering bud in 'Hamlin' during 1999) (Table 2-12). Statistically equivalent groupings were established for the phenological parameters flowers-per-shoot and flowers-per-

flowering-bud for both cultivars during the 1999 and 2000 season (Tables 2-13 to 2-16). In all situations the number of flowers per shoot was increased as the buds were exposed to more inductive conditions. The effect of induction levels on flowers per flowering bud was not significant in 'Hamlin' during 1999. Although induction levels, in general increased the number of flowers per flowering bud for both seasons of 'Valencia,' and for the 2000 season of 'Hamlin,' this effect was not consistent across treatments.

For the same level of induction, the presence of crop load diminished the development of flowers per shoot for both cultivars and seasons (Tables 2-12 to 2-16). The presence of crop load also diminished the number of flowers per flowering bud during the 2000 season for both cultivars.

Effects of different induction levels on floral distribution by bud position along the shoot

The effects of induction and crop load treatments on the distribution of flowers along the shoot were more clearly observed in positions near the apex of the shoot (Table 2-17). In general, more flowers developed as the winter progressed and more flower induction conditions occurred. In addition, more flowers developed in more apical positions over more basal positions (Tables 2-18 to 2-21 and Figs. 2-3 and 2-4).

There was a decreasing gradient according to the induction level. Some floral development occurred in buds at the apical position at the lowest induction levels. Positions farther from the apex decreased the number of flowers that developed considerably. Furthermore, positions farther from the apex only developed floral buds at the highest induction levels.

Analysis 3. Effect of Weather Data on the Phenological Response of 'Valencia' and 'Hamlin' Trees

Vegetative buds per shoot

In both 'Valencia' and 'Hamlin', increases in the number of vegetative buds per shoot were inversely associated with increases in the number of hours from 25 to 30°C (Table 2-22). Other temperature ranges were not associated with the variation in the number of vegetative buds per shoot by this statistical method. The presence of fruit was associated with the number of vegetative buds only for 'Hamlin'. Warmer temperatures before the treatment date were associated with a decrease in vegetative sprouting. Presence of crop load was associated with an increase in vegetative sprouting. The resulting equations accounted for 60.5% and 28.8% of the variability in vegetative buds for 'Hamlin' and 'Valencia,' respectively.

Flowering buds per shoot

In 'Hamlin,' the number of flowering buds per shoot was significantly increased with increasing total number of hours below 15°C. For 'Valencia,' the most important temperature range for increased flowering was from 10 to 15°C. A significant decrease in the number of flowering buds per shoot was observed with the total number of hours above 30°C. For both cultivars, the presence of fruit was also associated with the decrease in total number of flowering buds per shoot (Table 2-23). The resulting equations accounted for 79.3% and 87.7% of the variability of flowering buds per shoot for 'Hamlin' and 'Valencia,' respectively.

Flowers per flowering bud

For 'Hamlin' and 'Valencia', an increase in the number of hours in the 10-15°C range was associated with an increase in the number of flowers in the inflorescences,

while the presence of fruit was inversely associated with this parameter (Table 2-24). The resulting equations accounted for only 18.1% of the variability for 'Hamlin' but 62.1% for 'Valencia' branches.

Flowers per shoot

The presence of crop and the occurrence of temperatures above 30°C for 'Hamlin' and from 25-30°C for 'Valencia' were associated with a decrease in the number of flowers per shoot. The resulting equations accounted for 75.3% of the variability in the number of flowers per shoot of 'Hamlin' branches, and 84.1% of 'Valencia' (Table 2-25).

Discussion

Analysis 1. Evaluation of Phenological Response (Bud Sprouting and Flowering Parameters) of 'Valencia' and 'Hamlin' Trees as Affected by Season and Crop Load

Bud sprouting parameters

Increased total bud sprouting during the 2000 season in comparison with the 1999 season can be attributed to the enhancement of floral bud sprouting caused by increased inductive conditions. This enhancement of floral bud sprouting during the 2000 season (when compared to the 1999 season) may be explained by an increase in cool temperatures from the 1999 to the 2000 season (Table 2-26). The 2000 winter had more hours in the low temperature ranges. From 1 Oct 1999 to 1 Mar 2000 there were 1012 h below 15°C, in comparison to the 612 h of 1999. This presumably caused a longer and more intense low-temperature stress in the trees, which in turn caused an increase in induction of reproductive buds. These results agree with previous reports by Furr and Armstrong (1956) and Ginestar and Castel (1996) that have concluded that an increase in intensity and duration of the inductive stress influences the intensity of citrus flowering. In addition, a greater number of hours in the high temperatures ranges during the 1999

season could have caused reduced floral bud sprouting. As this effect was statistically significant only in branches without crop load, it suggests that the presence of crop load reduces the effectiveness of low temperatures to cause floral induction, or that the presence of fruit increases the low temperature requirements for floral induction.

The effects of crop load on bud sprouting were inconsistent. During the 1999 season, crop load did not have a significant effect on any of the sprouting parameters of 'Hamlin' branches, but the effects were significant during the 2000 season. Crop load reduced the sprouting of flowering buds across seasons and cultivars. However these results were not consistent in their significance. Similarly, crop load presence seemed to increase the number of vegetative buds per shoot in all situations, however this increment was only statistically significant for 'Hamlin' branches during the 2000 season. This response contrasts with the reports by Goldschmidt and Monselise (1972) and Reuther (1973) which found that fruit has an inhibitory effect on the production of vegetative flushes. Inconsistencies in the significance of the effect of seasonal and crop load might be attributable to variability in the data rather than the null effect of crop load during those seasons.

Effect of season and crop load on flowering phenology

The extension of the flowering period of both seasons was significantly different from the 1999 to the 2000 season (the flowering period of the 1999 season extended for 37 d., in comparison with 9 d. for the 2000 season). The extended anthesis of 1999 suggests the occurrence of multiple flushes of flowering buds, which consists of groups of buds, or branches flowering successively (waves of bloom). The extended flowering period may be explained by the differences in the weather patterns of both years. The 1998-1999 winter season was warmer than the 1999-2000 season (see above), with

considerably fewer hours in the low temperature ranges (below 15°C) during 1998-1999 season, and more high temperatures during the same period of time (Table 2-22). While low temperature stress (inductive conditions) was occurring during the winter of 1998-1999, warmer temperatures were also causing periodic bud sprouting in different branches. Flowering buds sprouted as their induction requirement were satisfied and growth conditions occurred. More constant low temperatures during the 1999-2000 winter season caused branches to remain inactive throughout the winter inductive conditions. Once the induction requirement was satisfied and warmer conditions occurred, buds sprouted and caused relatively simultaneous flowering.

Seasonal and crop load effects on flowering parameters were inconsistent across cultivars. Seasonal increments from the 1999 to 2000 season in all flowering parameters were observed for both cultivars and seasons. However, this increase was considered inconsistently significant between the two cultivars and seasons. This difference in significance may be due to data dispersion rather than a null seasonal effect. Similarly the presence of crop diminished the majority of the flowering parameters. However, this effect was not sufficiently constant across replications to result in consistently significant differences.

Floral distribution by shoot age (summer vs. spring)

The effect of shoot age (summer vs. spring shoots) was similar across cultivars and seasons. From the totality of the marked shoots, the majority of the flowers developed on buds from the previous summer flush of growth. Equal numbers of spring shoots developed fewer flowers per shoot than summer shoots. Thus, the age of the shoots had a significant effect on the development of flowers. An older shoot is less likely to induce floral buds than a younger shoot (Guardiola, 1981). Although this effect

is important to determine the different inductive conditions needed for summer and spring shoots, the proportion of flowers may not be extrapolated to the whole tree level as shoots of different ages are not present in the same proportions in citrus trees. Unpublished observations by Dr. L. G. Albrigo (personal communication) indicate that more spring than summer flush occurs in the tree. Although these increased numbers are not likely to make up for greater number of flowers, they should be enough to alter the percentage distribution of flowers developing from summer and spring shoots (Guardiola, 1981).

In both cultivars, floral distribution by position along the shoot (for summer and spring shoots) showed a marked apical tendency. In all situations the majority of the flowers were located at or near the apex of the shoot (positions 1 through 3), and decreased gradually towards the more basal positions. As fewer flowers developed in spring shoots flower development in more basal buds of these older shoots was negligible.

Analysis 2. Effect of Different Induction Levels on the Phenological Response of 'Valencia' and 'Hamlin' Trees

Effects of different induction levels and crop load on phenological parameters

Low temperature inductive conditions have a contrasting effect on the sprouting of reproductive and vegetative flushes. In general low temperature inductive conditions causes the bud to make the transition from the vegetative to reproductive stages. The sprouting of vegetative buds is therefore reduced in favor of the sprouting of reproductive buds.

Differences in the significance of the effects of crop load on bud sprouting also suggest that the effect of crop load is stronger in 'Hamlin' than in 'Valencia' branches.

The lack of significance of the effect of crop load on total bud sprouting of 'Hamlin' branches was masked by the fact that crop load increased the number of vegetative buds and decreased the number of reproductive buds.

In this study, the main parameter for floral intensity is the number of flowers per total number of shoots able to bear flowers (flowers-per-shoot parameter). This parameter was significantly increased as the induction levels progressed through the winter time. The most intense flowering was achieved at the highest induction levels (control and latest treatments), and relatively minor flowering occurred at the lowest induction levels. Increased induction levels had a significant effect on floral intensity primarily by increasing the number of reproductive buds per shoot. Although induction levels also affected the number of flowers per reproductive bud (number of flowers in the inflorescences) this effect was less systematic. In general greater induction levels also caused more flowers per sprouting bud. However, there were a number of cases in which branches that had been exposed to a lesser amount of inductive conditions developed more flowers than those exposed to more inductive conditions, and viceversa. Induction levels even had a non-significant effect on the number of flowers per flowering bud in 'Hamlin' branches during the 1999 season.

As induction levels progressed, the presence of crop load had a significant effect in the development of flowers per shoot for both cultivars and seasons. This effect also points out that crop load reduces the sensitivity of the buds to inductive conditions, or to the fact that more inductive conditions are needed to overcome the potential negative effect of crop load on floral induction.

Effects of different induction levels on the floral distribution by bud position along the shoot

Induction levels and crop load treatments had a significant effect on the reproductive growth of buds at different positions along the shoot. These significant effects indicate that their induction requirement is different according to their position. In all cases the easiest buds to induce were located near the apex of the shoots, and the most difficult were located far from the apex. Therefore, flowering response to the same inductive conditions gradually diminished as the buds were located more distant from the apex. Buds in more basal positions were more difficult to induce to develop flowers and their reproductive sprouting was limited to the highest inductive conditions. The presence of crop diminished the magnitude of the response but the overall response was the same.

Analysis 3. Effect of Weather on the Phenological Response of 'Valencia' and 'Hamlin' Trees

Vegetative buds per shoot

The statistical equation that related vegetative buds per shoot to number of hours of temperature in five-degree increments associated an increase in the number of hours between 25-30°C to a decrease in the sprouting of vegetative buds (Table 2-24). This model indicates that warm winters and crop load decrease the sprouting of vegetative buds. A potential drawback of this model is that it does not indicate whether or not low temperatures are needed for the sprouting of vegetative buds. The fact that no low temperature ranges were considered significant by the statistical model does not necessarily mean that low temperatures are not needed for the sprouting of vegetative buds. It is possible that the low-temperature requirement for the sprouting of vegetative

buds was fully satisfied in both years and therefore it was not a factor in the multiple regression analysis.

For 'Hamlin' branches, the presence of crop load was significantly associated with an increase in the sprouting of vegetative buds. Although the same increasing effect could be seen for 'Valencia,' the effect of the crop load variable did not cause a significant effect on the sprouting of vegetative buds. These cultivar differences in crop load effect seem to suggest that developing fruitlets may have a milder effect for 'Valencia' in comparison with 'Hamlin' branches. Another possibility is related to the fact that 'Hamlin' trees usually carry a heavier load of fruit than 'Valencia.'

Flowering buds per shoot

The model that relates the sprouting of flowering buds to the number of hours in the different temperature ranges resulted in low temperatures being significantly associated with increasing numbers of flowering buds (Table 2-25). For 'Hamlin,' significant low temperatures were those below 15°C. A similar range was obtained for 'Valencia' branches, in which the number of hours in the range between 10-15°C also significantly increased the number of reproductive buds. Therefore, for both cultivars, a colder winter before the interruption of induction was associated with an increase in the total number of flowering buds per shoot. In addition, high temperatures decreased the number of reproductive buds, but only for 'Valencia' branches. Although in the statistical analyses only these ranges in the low temperature range were statistically significant for the sprouting of reproductive buds, other ranges would be expected to have an effect on induction levels. The fact that other ranges were not statistically significant may be related to low number of hours or low variability in the other ranges. Another

possibility is that the effect of the most effective temperature range may have hidden the effects of other ranges, particularly if they varied similarly to the most effective range.

In agreement with the results of other published studies (Bellows and Morse, 1986; Ben Mechlia and Carroll, 1989; Lovatt et al., 1984), temperature was considered one of the most important environmental conditions that affected citrus reproductive response. Hours in the low temperature ranges (10-15°C range) significantly increased flower response of citrus buds, while high temperatures (above 25°C for 'Hamlin' and 30°C for 'Valencia') tended to inhibit the same response. Based on this equation, for both cultivars, a colder winter is associated with an increase in the total number of flowering buds per shoot. In both cultivars, the presence of fruit reduced the number of flowering buds per shoot.

As with the sprouting of vegetative buds, the presence of crop load reduced the sprouting of reproductive buds of both cultivars. Differences in the effects of crop load on the sprouting of reproductive buds may be inferred by the parameter estimate of crop load and their partial R^2 . Crop load on 'Hamlin' branches seemed to have a stronger effect than 'Valencia' branches on the sprouting of flowering buds. Crop load effects remained significant regardless of the fact that 'Hamlin' fruits were harvested during the study and that 'Valencia' fruits remained throughout both flowering seasons.

Flowers per flowering bud

The statistical model that relates the number of flowers per flowering bud associates low temperatures (in the 10-15°C range) and crop load as important factors affecting the number of flowers per flowering bud (Table 2-26). Based on this model, a more extended winter (with higher number of hours in the 10-15°C range) would cause not only the development of a greater number of flowers in each flowering bud, but also

an increase in the sprouting of reproductive buds. Therefore, an extended winter would cause an increase in the intensity of flowering. This result goes in accordance with the commonly accepted notion that the duration, intensity, and time course of the stress directly influence the intensity of citrus flowering (Furr and Armstrong, 1956). This result specifically suggests that the 10-15°C range is the most effective temperature range affecting the intensity of citrus flowering.

Flowers per shoot.

The equation resulting for the total number of flowers per shoot as a function of temperature and crop load also suggests that floral intensity (given in terms of flowers per shoot) increase with low temperatures, and diminish with the presence of crop load and the occurrence of high temperatures (Table 2-27). The effect of high temperatures may be related to the decrease in the sprouting of flowering buds.

Conclusions

Citrus flowering is a complicated process in which a number of factors intervene to produce the phenological response of citrus trees. Under Florida conditions, a number of factors determine the phenological response of an individual bud to environmental conditions. These factors include the cultivar, position of the bud along the shoot, age of the shoot, level of induction, and presence or absence of crop load on the branch.

Induction requirements varied according to the position of the bud along the shoot. Buds at the apical positions sprouted more readily than those located far from it. Buds at the more basal positions sprouted predominantly at the highest induction levels.

For the same inductive conditions, different numbers of flowers developed depending on the age of the shoots. For an equal number of shoots, more flowers developed from younger (summer shoots) over older shoots (spring shoots).

The presence or absence of fruit (or recently removed fruit) had a significant effect on the phenological response of both cultivars. Crop load favored the sprouting of vegetative over reproductive buds. Cultivar differences in the intensity of the effect of crop load on flowering phenology are discussed. Although flowering phenology of 'Hamlin' seemed to be more strongly affected by crop load than 'Valencia'. These differences may be caused by differences in the strength of the effect of the fruit, by the presence of more crop load (increase in fruit numbers) in 'Hamlin' over 'Valencia', or by an increased number of flowers that could be affected by the presence of crop. As the present study only evaluated the effects of the presence or absence of fruit in a branch, to quantify the effects of crop load on the flowering response it would be necessary to evaluate flowering response at different levels of crop load (e.g. low, medium, or high).

Within the scope of this study, low temperatures and presence of crop were the two most important factors affecting floral intensity. The intensity of citrus flowering is a function of the number of sprouting buds with reproductive growth, and the number of flowers that develop from each flowering bud. The effect of increased low temperature inductive conditions on enhanced floral intensity was explained primarily by an increase in the number of reproductive buds, and increasing effect on the number of flowers per reproductive bud. The reducing effect of crop load on the reproductive response may be explained in two ways. Crop load increases the low temperature inductive requirement for the development of floral buds, or, for similar inductive conditions, crop load decreases the reproductive response of floral buds.

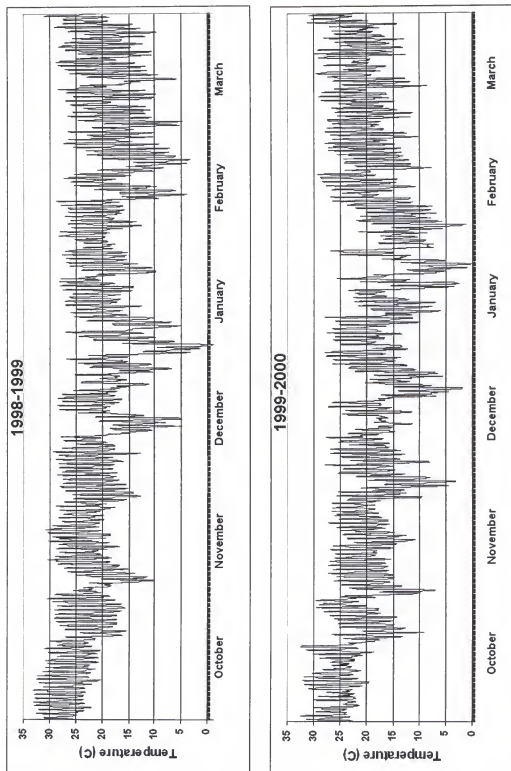


Fig. 2-1. Local temperature data from the Florida Automated Weather Network system for the Lake Alfred station during the 1998-1999 and 1999-2000 seasons.

Table 2-1 Description of induction and crop load treatments (6 reps.) of sweet orange trees for Valencia and Hamlin during the 1998-1999 and 1999-2000 seasons

Season	Cultivar	Trt. Id. ^z	Date ^y	Crop load levels ^x	Cultivar	Trt. Id.	Date	Crop load levels
1999	Hamlin	1	3 Nov	With	Valencia	1	3 Nov	With
				Without				Without
		2	17 Nov	With		2	17 Nov	With
				Without				Without
		3	4 Dec	With		3	4 Dec	With
				Without				Without
		4	18 Dec	With		4	18 Dec	With
				Without				Without
		5	4 Jan	With		5	4 Jan	With
1999	Hamlin			Without				Without
		6	19 Jan	With		6	19 Jan	With
				Without				Without
		7	2 Feb	With		7	2 Feb	With
				Without				Without
		8	16 Feb	With		8	16 Feb	With
				Without				Without
		Cont.	-	With		Cont.	-	With
				Without				Without
2000	Hamlin	1	3 Nov	With	Valencia	1	3 Nov	With
				Without				Without
		2	18 Nov	With		2	18 Nov	With
				Without				Without
		3	3 Dec	With		3	3 Dec	With
				Without				Without
		4	16 Dec	With		4	16 Dec	With
				Without				Without
		5	3 Jan	With		5	3 Jan	With
2000	Hamlin			Without				Without
		6	18 Jan	With		6	18 Jan	With
				Without				Without
		7	4 Feb	With		7	4 Feb	With
				Without				Without
		Cont.	-	With		Cont.	-	With
				Without				Without

^z Treatment identifier refers to the date in which floral induction was interrupted.

^y Date of interruption of induction by the girdling and defoliation technique

^x Crop load levels refers to the presence or absence of fruit on the branch

Table 2-2. Comparison of bud sprouting parameters of 'Hamlin' sweet orange trees from the 1999 to the 2000 seasons (rows), and with and without crop load (columns)

Variable	Crop Load ^z	Mean 1999	Mean 2000	t value ^y	Sign. ^x
Sprouting buds per shoot	Without	1.72	2.70	-2.73	*
	With	1.55	2.14	-1.81	NS
	t value ^w	0.47	1.61		
	Sign.	NS	NS		
Vegetative buds per shoot	Without	0.10	0.14	-0.66	NS
	With	0.24	0.59	-2.62	*
	t value	-1.39	-4.84		
	Sign.	NS	**		
Flowering buds per shoot	Without	1.61	2.56	-2.51	*
	With	1.31	1.55	-0.67	NS
	t value	0.82	2.74		
	Sign.	NS	*		

^z Selected branches with or without crop load (developing fruitlets)

^y t-value for the comparison between average values between the 1999 and the 2000 season

^x *, **, and NS. Significant t test at $P \leq 0.05$ and 0.01 and non-significant, respectively.

^w t-value for the comparison between average values at the two different crop loads.

Table 2-3. Comparison of bud sprouting parameters of 'Valencia' sweet orange trees from the 1999 to the 2000 seasons (rows), and with and without crop load (columns)

Variable	Crop Load ^z	Mean 1999	Mean 2000	t value ^y	Sign. ^x
Sprouting buds per shoot	Without	1.27	2.48	-3.23	**
	With	1.16	1.80	-1.37	NS
	t value ^w	0.52	1.31		
	Sign. ^x	NS	NS		
Vegetative buds per shoot	Without	0.46	0.37	0.83	NS
	With	0.74	0.54	0.86	NS
	t value	-1.85	-0.82		
	Sign. ^x	NS	NS		
Flowering buds per shoot	Without	0.81	2.12	-3.44	**
	With	0.42	1.26	-1.74	NS
	t value	2.41	1.55		
	Sign. ^x	*	NS		

^z Selected branches with or without crop load (developing fruitlets)

^y t-value for the comparison between average values between the 1999 and the 2000 season

^x *, **, and NS. Significant t test at $P \leq 0.05$ and 0.01 and non-significant, respectively.

^w t-value for the comparison between average values at the two different crop loads.

Table 2-4. Comparison of flowering parameters of 'Hamlin' sweet orange trees from the 1999 to the 2000 seasons (rows), and with and without crop load (columns)

Variable	Crop Load ^z	Mean 1999	Mean 2000	t value ^y	Sign. ^x
Flowers per shoot	Without	4.56	8.26	-2.45	*
	With	3.74	5.80	-0.97	NS
	t value ^w	0.65	1.11		
	Sign. ^x	NS	NS		
Flowers per flowering bud	Without	2.86	3.18	-0.84	NS
	With	2.99	3.26	-0.38	NS
	t value ^w	-0.29	-0.13		
	Sign. ^x	NS	NS		
Flowers per sprouting bud	Without	2.59	3.00	-1.13	NS
	With	2.44	1.99	1.04	NS
	t value ^w	0.33	2.77		
	Sign. ^x	NS	*		

^z Selected branches with or without crop load (developing fruitlets)^y t-value for the comparison between average values between the 1999 and the 2000 season (horizontal comparisons)^x *, **, and NS. Significant t test at $P \leq 0.05$ and 0.01 and non-significant, respectively.^w t-value for the comparison between average values at the two different crop loads (vertical comparisons)

Table 2-5. Comparison of flowering parameters of 'Valencia' sweet orange trees from the 1999 to the 2000 seasons (rows), and with and without crop load (columns)

Variable	Crop Load ^z	Mean 1999	Mean 2000	t value ^y	Sign. ^x
Flowers per shoot	Without	1.72	5.72	-2.05	NS
	With	0.68	3.38	-2.47	*
	t value ^w	3.4	1.05		
	Sign. ^x	**	NS		
Flowers per flowering bud	Without	2.17	3.45	-1.87	NS
	With	1.74	2.23	-1.26	NS
	t value ^w	1.51	2.21		
	Sign. ^x	NS	*		
Flowers per sprouting bud	Without	1.32	2.50	-2.34	*
	With	0.59	1.49	-2.27	*
	t value ^w	4.56	1.76		
	Sign. ^x	**	NS		

^z Selected branches with or without crop load (developing fruitlets)^y t-value for the comparison between average values between the 1999 and the 2000 season^x *, **, and NS. Significant t test at $P \leq 0.05$ and 0.01 and non-significant, respectively.^w t-value for the comparison between average values at the two different crop loads

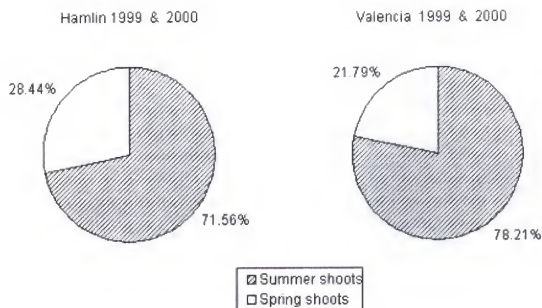


Fig. 2-2. Distribution of flowers by shoot age (summer vs. spring shoots)

Table 2-6. Cumulative percentages of floral distribution by bud position along the shoot of different age shoots (summer and spring) for 'Hamlin' and 'Valencia' trees combining the 1999 and 2000 seasons.

Bud Position ^z	Hamlin		Valencia	
	Summer ^y (%)	Spring ^x (%)	Summer (%)	Spring (%)
1	23.8	37.6	31.1	39.7
1 to 2	44.1	58.7	52.8	61.1
1 to 3	64.2	80.0	74.3	84.5
1 to 4	78.5	88.1	86.1	95.8
1 to 5	87.2	93.7	92.8	98.2
1 to 6	92.7	96.6	95.9	99.7
1 to 7	95.9	98.0	97.6	99.7
1 to 8	98.0	99.7	98.1	100
1 to 9	98.8	100	98.8	100
1 to 10	100	100	100	100

^z Bud position refers to the position along the shoot of the viable buds. Bud in Position 1 refers to the first viable bud located at the apex-most position

^y Cumulative percentage of flowers from summer shoots and their distribution according to their position along the shoot.

^x Cumulative percentage of flowers from spring shoots and their distribution according to their position along the shoot.

Table 2-7. Summary of analyses of variance of bud sprouting parameters (total sprouting, vegetative, and reproductive buds per shoot) for 'Hamlin' and 'Valencia' trees during the 1999 and 2000 seasons.

Variable	Sources of Variation	Hamlin 1999		Hamlin 2000		Valencia 1999		Valencia 2000	
		F-value ^z	Sign. ^y	F-value	Sign.	F-value	Sign.	F-value	Sign.
Sprouting buds per shoot	Model	3.23	**	2.42	**	1.61	NS	1.03	NS
	Induction	6.41	**	4.04	**				
	Crop load	0.52	NS	1.59	NS				
	Induction \times crop load	0.38	NS	0.92	NS				
Vegetative buds per shoot	Model	2.81	**	6.48	**	1.65	NS	2.06	*
	Induction	4.57	**	7.49	**			3.5	**
	Crop load	8.28	**	35.48	**			2.43	NS
	Induction \times crop load	0.36	NS	1.33	NS			0.56	NS
Reproductive buds per shoot	Model	6.83	**	6.54	**	5.5	**	2.79	**
	Induction	13.22	**	9.46	**	9.94	**	4.54	**
	Crop load	7.73	**	27.53	**	1.31	NS	5.83	*
	Induction \times crop load	0.33	NS	0.62	NS	1.58	NS	0.53	NS

^z F-value of the ANOVAS on the effect of induction and crop load treatments on sprouting parameters for each cultivar and season.

^y Statistical significance of the F-value: *, **, and NS corresponds to a significant F test at $P \leq 0.05$ and 0.01, and non-significant, respectively.

Table 2-9. Summary of sprouting parameters (vegetative and reproductive buds per shoot) for 'Hamlin' trees during the 2000 season.

Vegetative buds per total number of shoots					Reproductive buds per total number of shoots					
Hamlin w/ crop load			Hamlin no crop load		Hamlin w/ crop load			Hamlin no crop load		
Trt.	Mean ^y	D. ^x	Trt.	Mean	Trt.	Mean	D.	Trt.	Mean	D.
1	1.73	a	1	0.91	Cont.	1.55	a	Cont.	2.56	a
5	1.05	b	3	0.67	7	1.39	ab	7	1.77	b
2	1.04	b	6	0.6	5	0.94	abc	5	1.61	bc
4	0.99	b	4	0.48	3	0.87	abc	6	1.56	bc
6	0.91	b	7	0.45	2	0.82	abc	3	1.24	bc
7	0.79	b	2	0.45	6	0.74	bc	2	1.14	bc
3	0.7	b	5	0.4	4	0.39	c	1	1	c
Cont.	0.59	b	Cont.	0.14	1	0.38	c	4	0.91	c

^z Identifier of induction level (treatment). Treatment refers to the date in which floral induction was interrupted.^y Mean value for sprouting parameter at that induction level.^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter are not significantly different, $P \leq 0.05$.

Table 2-10. Summary of sprouting parameters (vegetative and reproductive buds per shoot) for 'Valencia' trees during the 1999 season

Vegetative buds per total number of shoots				Reproductive buds per total number of shoots			
Valencia w/ crop load		Valencia no crop load		Valencia w/ crop load		Valencia no crop load	
Trt. ^z	Mean ^y	D. ^x	Trt.	Mean	D.	Trt.	Mean
1	0.97	a	5	1.67	a	7	0.49
2	0.94	a	3	0.98	a	8	0.49
5	0.8	a	6	0.77	a	Cont.	0.42
4	0.78	a	2	0.67	a	6	0.41
6	0.75	a	4	0.65	a	5	0.26
Cont.	0.74	a	1	0.59	a	2	0.24
7	0.7	a	Cont.	0.46	a	4	0.12
3	0.47	a	8	0.42	a	3	0.06
8	0.41	a	7	0.4	a	1	0

^z Identifier of induction level (treatment). Treatment refers to the date in which floral induction was interrupted.

^y Mean value for sprouting parameter at that induction level

^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter are not significantly different, $P \leq 0.05$.

Table 2-11. Summary of 'Duncan' analyses of sprouting parameters (vegetative and reproductive buds per shoot) for 'Valencia' trees during the 2000 season.

Vegetative buds per total number of shoots					Flowering buds per total number of shoots										
Valencia w/ crop load					Valencia no crop load			Valencia w/ crop load					Valencia no crop load		
Trt. ^z	Mean ^y	D. ^x	Trt.	Mean	D.	Trt.	Mean	D.	Trt.	Mean	D.	Trt.	Mean	D.	
1	2.3	a	1	1.54	a	Cont.	1.26	a	Cont.	2.12	a	5	1.26	b	
5	1.24	b	4	1.46	ab	6	1.22	a	6	1.14	bc	4	1.13	bc	
3	1.16	b	3	0.86	ab	3	0.75	b	3	0.96	bc	2	0.78	bc	
6	1.15	b	6	0.8	ab	5	0.68	b	5	0.67	b	1	0.29	c	
2	1.04	b	5	0.79	ab	4	0.67	b	2	0.34	b				
4	1.04	b	2	0.67	ab	2	0.34	b	1	0.23	b				
Cont.	0.54	b	Cont.	0.37	b										

^z Identifier of induction level (treatment). Treatment refers to the date in which floral induction was interrupted.

^y Mean value for sprouting parameter at that induction level

^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter are not significantly different, $P \leq 0.05$.

Table 2-12. Summary of analyses of variance of flowering parameters (number of flowers per shoot, number of flowers per flowering bud, and number of flowers per sprouting bud) for 'Hamlin' and 'Valencia' trees during the 1999 and 2000 seasons

Variable	Sources of Variation	Hamlin 1999		Hamlin 2000		Valencia 1999		Valencia 2000	
		F-value ^z	Sign. ^y	F-value	Sign.	F-value	Sign.	F-value	Sign.
Flowers per shoot	Model	4.3	**	5.04	**	7.21	**	2.93	**
	Induction	8.34	**	6.59	**	11.49	**	4.94	**
	Crop load	4.18	*	25.24	**	9.22	*	4.73	*
	Induction × crop load	0.27	NS	0.59	NS	2.68	NS	0.62	NS
Flowers per Flowering bud	Model	1.21	NS	2.32	**	4.03	**	2.65	**
	Induction			3	**	7.24	**	4.62	**
	Crop load			11.05	**	3.88	NS	5.08	*
	Induction × crop load			0.38	NS	0.84	NS	0.26	NS
Flowers per Sprouting bud	Model	3.31	**	4.93	**	7.29	**	3.16	**
	Induction	5.71	**	4.71	**	11.87	**	5.14	**
	Crop load	3.81	NS	38.7	**	12.84	**	8.18	**
	Induction × crop load	0.84	NS	0.32	NS	2.02	NS	0.36	NS

^z F-value of ANOVAS on the effect of induction and crop load treatments on sprouting parameters for each cultivar and season.

^y Statistical significance of the F-value: *, **, and NS corresponds to a significant F test at $P \leq 0.05$ and 0.01, and non-significant, respectively.

Table 2-13. Summary of flowering parameters (total number of flowers per shoot, total number of flowers per flowering bud, and total number of flowers per sprouting bud) for Hamlin trees during the 1999 season.

Flowers per total number of shoots						Flowers per total number of flowering buds					
Hamlin w/ crop load			Hamlin no crop load			Hamlin w/ crop load			Hamlin no crop load		
Trt. ^z	Mean ^y	D ^x	Trt.	Mean	D.	Trt.	Mean	D.	Trt.	Mean	D.
8	3.92	a	8	5.02	a	3	3	a	2	3.32	a
Control	3.74	ab	Control	4.56	a	Control	2.99	a	3	2.95	ab
6	2.22	abc	7	2.95	ab	6	2.67	a	Control	2.86	ab
7	2	bc	6	2.92	ab	1	2.62	a	8	2.57	abc
2	1.5	c	4	2.27	b	8	2.59	a	7	2.56	abc
4	1.15	c	2	2.11	b	7	2.33	a	4	2.54	abc
3	1.12	c	3	1.87	b	2	2.27	a	6	2.49	abc
1	0.99	c	5	0.94	b	4	2.13	a	1	2.12	bc
5	0.97	c	1	0.71	b	5	1.91	a	5	1.71	c

^z Identifier of induction level (treatment) . Treatment refers to the date in which floral induction was interrupted.

^y Mean value for the flowering parameter at that induction level and crop load status

^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter (within columns) are not significantly different, $P \leq 0.05$.

Table 2-14. Summary of flowering parameters (flowers per shoot and number of flowers per flowering bud) for 'Hamlin' trees during the 2000 season.

Flowers per total number of shoots						Flowers per total number of flowering buds					
Hamlin w/ crop load			Hamlin no crop load			Hamlin w/ crop load			Hamlin no crop load		
Trt. ^z	Mean ^y	D. ^x	Trt.	Mean	D.	Trt.	Mean	D.	Trt.	Mean	D.
Control	4.47	a	Control	8.26	a	6	3.18	a	2	3.51	a
7	4.01	a	7	5.36	b	Control	2.77	ab	6	3.27	ab
5	2.63	ab	6	5.32	b	2	2.77	ab	3	3.22	ab
2	2.38	ab	5	5.04	bc	5	2.6	ab	Control	3.18	ab
3	2.3	ab	2	4.03	bc	7	2.5	ab	7	3.08	ab
6	2.3	ab	3	3.99	bc	3	2.49	ab	5	2.8	ab
4	1.06	b	1	3.42	bc	1	1.69	b	1	2.69	ab
1	0.94	b	4	1.99	c	4	1.67	b	4	2.29	b

^z Identifier of induction level (treatment) . Treatment refers to the date in which floral induction was interrupted.

^y Mean value for the flowering parameter at that induction level and crop load status

^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter (within columns) are not significantly different, $P \leq 0.05$.

Table 2-15. Summary of flowering parameters (total number of flowers per shoot, total number of flowers per flowering bud, and total number of flowers per sprouting bud) for 'Valencia' trees during the 1999 season.

Flowers per total number of shoots						Flowers per total number of flowering buds					
Valencia w/ crop load			Valencia no crop load			Valencia w/ crop load			Valencia no crop load		
Trt. ^z	Mean ^y	D. ^x	Trt.	Mean	D.	Trt.	Mean	D.	Trt.	Mean	D.
8	0.77	a	Control	1.72	a	5	1.83	a	Control	2.17	a
Control	0.68	ab	7	0.91	b	Control	1.74	a	7	2.08	ab
7	0.48	abc	8	0.9	b	6	1.24	ab	5	1.96	ab
6	0.48	abc	6	0.76	bc	4	1.18	ab	8	1.93	ab
5	0.45	abc	5	0.48	bcd	8	1.16	ab	6	1.62	abc
2	0.36	abc	4	0.3	cd	2	1.11	ab	4	1.19	abc
4	0.21	bc	3	0.28	cd	7	0.99	abc	3	0.95	bcd
3	0.06	c	2	0.06	d	3	0.49	c	2	0.72	dc
1	0	c	1	0	d	1	0	c	1	0	d

^z Identifier of induction level (treatment). Treatment refers to the date in which floral induction was interrupted.

^y Mean value for the flowering parameter at that induction level and crop load status

^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter (within columns) are not significantly different, $P \leq 0.05$.

Table 2-16. Summary of flowering parameters (total number of flowers per shoot, total number of flowers per flowering bud, and total number of flowers per sprouting bud) for 'Valencia' trees during the 2000 season.

Flowers per total number of shoots						Flowers per total number of flowering buds					
Valencia w/ crop load			Valencia no crop load			Valencia w/ crop load			Valencia no crop load		
Trt. ^z	Mean ^y	D. ^x	Trt.	Mean	D.	Trt.	Mean	D.	Trt.	Mean	D.
Cont.	3.38	a	Cont.	6.36	a	Cont.	2.2	a	Cont.	2.94	a
6	2.49	ab	5	2.49	b	6	1.67	ab	2	2.32	a
4	1.71	ab	6	2.4	b	5	1.61	ab	6	1.94	a
3	1.69	ab	3	2.31	b	2	1.4	ab	3	1.79	a
5	1.32	ab	4	2.27	b	3	1.39	ab	5	1.76	a
2	0.49	b	2	1.83	b	4	0.99	ab	4	1.73	a
1	0.1	b	1	0.35	b	1	0.37	b	1	0.53	b

^z Identifier of induction level (treatment) . Treatment refers to the date in which floral induction was interrupted.

^y Mean value for the flowering parameter at that induction level and crop load status

^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter (within columns) are not significantly different, $P \leq 0.05$.

Table 2-17. Summary of analyses of variance for the effect of induction and crop load treatments on the development of flowers per shoot for 'Hamlin' and 'Valencia' trees during the 1999 and 2000 seasons

Cultivar	Pos. 1 F ^z Sign.	Pos. 2 F Sign.	Pos. 3 F Sign.	Pos. 4 F Sign.	Pos. 5 F Sign.	Pos. 6 F Sign.	Pos. 7 F Sign.	Pos. 8 F Sign.	Pos. 9 F Sign.	Pos. 10 F Sign.
Hamlin 1999										
Ind. Levels	8.34 **	12.42 **	8.91 **	6.44 **	4.53 **	3.24 **	1.68 NS	1.3 NS	2.48 *	0.62 NS
Crop Load	4.18 *	3.14 *	7.69 **	3.85 NS	1.98 *	0.77 NS	0.79 NS	0.12 NS	2.09 NS	0.61 NS
Ind. levels x Crop Load	0.27 NS	0.14 NS	0.5 NS	0.75 NS	0.26 NS	0.92 NS	0.49 NS	0.7 NS	0.78 NS	0.72 NS
Hamlin 2000										
Ind. Levels	7.43 **	11.33 **	6 **	4.11 **	2.71 *	2.2 *	2.4 *	1.95 NS	1.75 NS	0.86 NS
Crop Load	26.17 **	12.16 **	23.87 **	15.34 **	33.2 **	10.83 **	8.99 **	7.7 **	4.3 *	2.96 NS
Ind. levels x Crop Load	0.58 NS	0.83 NS	1.31 NS	0.83 NS	0.55 NS	1.17 NS	1.11 NS	0.98 NS	0.65 NS	0.48 NS
Valencia 1999										
Ind. Levels	11.49 **	9.27 **	4.48 **	4.79 **	3.27 **	2.27 *	2.12 *	0.67 NS	1.74 NS	
Crop Load	9.22 **	3.78 NS	4.58 *	4.17 *	5.2 *	2.57 NS	3.21 NS	1.23 NS	1.9 NS	
Ind. levels x Crop Load	0.68 NS	1.78 NS	1.11 NS	1.34 NS	1.24 NS	0.99 NS	1.93 NS	0.53 NS	1.48 NS	
Valencia 2000										
Ind. Levels	4.94 **	5.72 **	2.61 *	4.73 **	2.83 *	2.2 NS	1.69 NS	1.86 NS	1.11 NS	3.26 NS
Crop Load	4.73 *	4.4 *	2.64 NS	6.81 *	2.16 NS	0.97 NS	0.01 NS	0.11 NS	0.66 NS	5.68 NS
Ind. levels x Crop Load	0.62 NS	0.36 NS	0.74 NS	0.67 NS	0.45 NS	1.73 NS	0.84 NS	0.14 NS	1.95 NS	3.73 NS

^z F-value of the ANOVAS on the effect of induction and crop load treatments on flower development by bud position along the shoot.

^y Statistical significance of the F-value: *, **, and NS corresponds to a significant F test at $P \leq 0.05$ and 0.01, and non-significant, respectively.

Table 2-18. Summary of number of flowers per shoot by position along the shoot of Hamlin branches during the 1999 season

Position 1			Position 2			Position 3			Position 4			Position 5		
Trt.	Avg. ^y	D. ^x	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.
8	4.47	a	8	1.55	a	Control	0.94	a	8	0.94	a	8	0.59	a
Control	4.18	a	Control	1.27	a	8	0.93	a	Control	0.76	ab	Control	0.49	ab
6	2.54	b	6	0.92	b	6	0.64	b	6	0.51	bc	7	0.26	bc
7	2.51	b	7	0.90	b	7	0.61	b	7	0.51	bcd	6	0.22	c
2	1.81	bc	2	0.72	bc	4	0.38	bc	2	0.43	cd	4	0.20	c
4	1.71	bc	4	0.57	bcd	2	0.36	bc	4	0.38	cd	2	0.17	c
3	1.46	bc	3	0.55	bcd	3	0.35	bc	3	0.34	cd	1	0.11	c
5	0.95	c	5	0.39	cd	5	0.26	c	1	0.23	cd	3	0.11	c
1	0.84	c	1	0.20	d	1	0.12	c	5	0.20	d	5	0.06	c

Position 6			Position 7			Position 8			Position 9			Position 10		
Control	0.30	a	Control	0.17	a	Control	0.12	a	Control	0.11	a	Control	0.01	a
8	0.26	ab	8	0.11	ab	8	0.06	ab	3	0.03	b	8	0.00	a
6	0.18	abc	7	0.07	ab	1	0.06	ab	4	0.02	b	6	0.00	a
7	0.12	bc	4	0.06	ab	7	0.05	ab	8	0.02	b	1	0	a
1	0.08	c	6	0.04	ab	3	0.02	ab	6	0.01	b	3	0	a
4	0.08	c	2	0.04	ab	6	0.02	ab	7	0.00	b	5	0	a
2	0.07	c	3	0.03	b	4	0.02	ab	1	0	b	2	0	a
3	0.04	c	1	0.03	b	2	0.01	ab	5	0	b	7	0	a
5	0.03	c	5	0.02	b	5	0	b	2	0	b	4	0	a

^z Identifier of induction level (treatment). Treatment refers to the date in which floral induction was interrupted.^y Mean value of flowers per shoot for that position and induction level^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter (within columns) are not significantly different, $P \leq 0.05$.

Table 2-19. Summary of number of flowers per shoot by position along the shoot of Hamlin branches during the 2000 season

Position 1			Position 2			Position 3			Position 4			Position 5		
Trt. ^z	Avg. ^y	D. ^x	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.
Control	6.48	a	Control	2.36	a	Control	1.29	a	Control	1.07	a	Control	0.58	a
7	4.68	b	7	1.56	b	7	0.99	ab	6	0.9	a	3	0.5	ab
6	4.11	b	5	1.41	bc	6	0.73	bc	2	0.89	a	6	0.5	ab
5	3.99	b	6	1.2	bcd	3	0.65	bc	7	0.88	a	7	0.47	ab
3	3.15	bc	2	0.9	cde	5	0.61	bc	5	0.79	ab	5	0.41	ab
2	3.07	bc	3	0.84	de	2	0.51	c	3	0.75	ab	2	0.36	abc
1	1.97	c	4	0.61	e	1	0.46	c	1	0.45	bc	1	0.28	bc
4	1.44	c	1	0.59	e	4	0.3	c	4	0.24	c	4	0.12	c

Position 6			Position 7			Position 8			Position 9			Position 10		
Control	0.46	a	6	0.29	a	Control	0.16	a	Control	0.15	a	Control	0.04	a
6	0.35	ab	Control	0.28	ab	7	0.12	ab	7	0.07	ab	5	0.03	a
7	0.35	ab	7	0.2	abc	5	0.11	ab	6	0.06	ab	7	0.02	a
5	0.31	ab	5	0.2	abc	6	0.07	ab	5	0.04	ab	3	0.01	a
2	0.25	ab	3	0.09	abc	2	0.06	ab	2	0.03	b	1	0	a
3	0.22	ab	2	0.08	bc	3	0.06	ab	4	0.03	b	2	0	a
1	0.13	b	4	0.04	c	4	0	b	1	0.02	b	4	0	a
4	0.11	b	1	0.04	c	1	0	b	3	0.02	b	6	0	a

^z Identifier of induction level (treatment). Treatment refers to the date in which floral induction was interrupted.^y Mean value of flowers per shoot for that position and induction level.^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter (within columns) are not significantly different, $P \leq 0.05$.

Table 2-20. Summary of number of flowers per shoot by position along the shoot of Valencia branches during the 1999 season

Position 1			Position 2			Position 3			Position 4			Position 5		
Trt. ^z	Avg. ^y	D. ^x	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.
Control	1.24	a	Control	0.56	a	Control	0.28	a	Control	0.21	a	Control	0.1	a
8	0.81	b	8	0.43	ab	8	0.17	ab	7	0.17	a	7	0.1	ab
7	0.76	b	7	0.33	b	7	0.17	ab	6	0.16	a	6	0.1	ab
6	0.61	bc	5	0.26	bc	6	0.10	bc	8	0.15	a	8	0.0	b
5	0.47	bcd	6	0.25	bc	2	0.08	bc	5	0.12	ab	4	0.0	b
4	0.26	cde	4	0.14	cd	5	0.07	bc	3	0.04	bc	3	0	b
2	0.20	de	3	0.10	cd	4	0.06	bc	4	0.03	bc	5	0	B
3	0.18	de	2	0.10	cd	3	0.03	bc	2	0.02	bc	1	0	B
1	0	e	1	0	d	1	0	c	1	0	c	2	0	B

Position 6			Position 7			Position 8			Position 9			Position 10		
Control	0.05	a	Control	0.02	a	Control	0.01	a	Control	0	a	Control	0	a
6	0.03	ab	6	0.01	ab	7	0	a	1	0	b	1	0	a
8	0.03	ab	8	0	b	2	0	a	2	0	b	2	0	a
7	0.03	ab	7	0	b	1	0	a	3	0	b	3	0	a
5	0.01	ab	1	0	b	4	0	a	4	0	b	4	0	a
3	0	b	5	0	b	5	0	a	5	0	b	5	0	a
4	0	b	2	0	b	6	0	a	6	0	b	6	0	a
2	0	b	3	0	b	3	0	a	7	0	b	7	0	a
1	0	b	4	0	b	8	0	a	8	0	b	8	0	a

^z Identifier of induction level (treatment). Treatment refers to the date in which floral induction was interrupted.^y Mean value of flowers per shoot for that position and induction level.^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter (within columns) are not significantly different, $P \leq 0.05$.

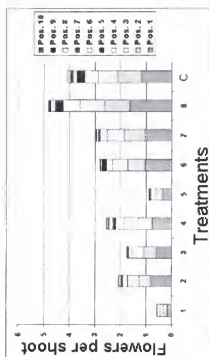
Table 2-21. Summary of number of flowers per shoot by position along the shoot of Valencia branches during the 2000 season

Position 1			Position 2			Position 3			Position 4			Position 5		
Trt. ^z	Avg. ^y	D. ^x	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.
Control	4.77	a	Control	1.38	a	Control	0.99	a	Control	1.11	a	Control	0.64	a
6	2.45	b	6	0.9	ab	5	0.62	ab	3	0.65	ab	6	0.29	ab
3	1.98	bc	4	0.72	b	3	0.47	ab	4	0.55	bc	3	0.27	ab
4	1.95	bc	5	0.52	bc	6	0.45	ab	6	0.47	bc	5	0.19	b
5	1.85	bc	3	0.42	bc	4	0.45	ab	5	0.29	bc	2	0.18	b
2	1.16	bc	2	0.36	bc	2	0.26	b	2	0.26	bc	4	0.12	b
1	0.24	c	1	0.08	c	1	0.06	b	1	0.03	c	1	0.01	b

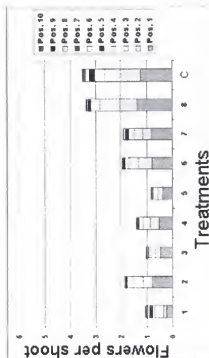
Position 6			Position 7			Position 8			Position 9			Position 10		
Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.	Trt.	Avg.	D.
Control	0.28	a	Control	0.15	a	Control	0.1	a	Control	0.02	a	Control	0.05	a
6	0.21	ab	6	0.1	ab	3	0.05	ab	6	0.02	a	5	0.01	b
5	0.11	ab	5	0.07	ab	5	0.02	ab	5	0.01	a	2	0	b
2	0.1	ab	4	0.05	ab	6	0	b	3	0.01	a	3	0	b
3	0.09	ab	1	0.03	ab	4	0	b	4	0	a	4	0	b
4	0.06	b	3	0.01	b	1	0	b	1	0	a	1	0	b
1	0.03	b	2	0	b	2	0	b	2	0	a	6	0	b

^z Identifier of induction level (treatment). Treatment refers to the date in which floral induction was interrupted.^y Mean value of flowers per shoot for that position and induction level^x Duncan multiple range test groupings, $P \leq 0.05$. Means with the same letter (within columns) are not significantly different, $P \leq 0.05$.

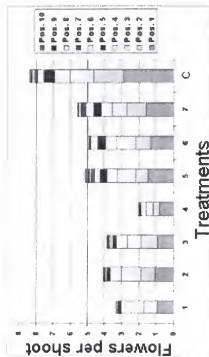
Hamlin 1999 No Crop Load



Hamlin 1999 With Crop Load



Hamlin 2000 No Crop Load



Hamlin 2000 With Crop Load

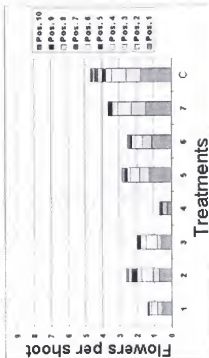
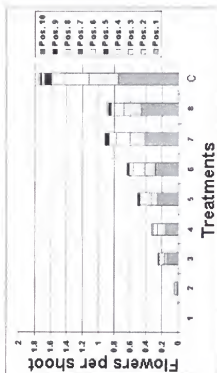
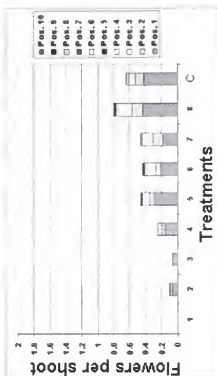


Fig. 2-3. Effects of different induction levels and crop load treatments on the distribution of flowers by bud position along the shoot of 'Hamlin', sweet orange trees during the 1999 and 2000 seasons. Data are presented as flowers per shoot.

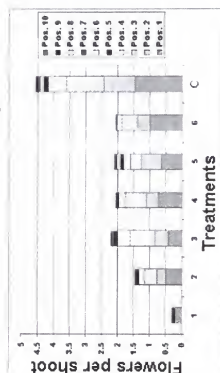
Valencia 1999 No Crop Load



Valencia 1999 With Crop Load



Valencia 2000 No Crop Load



Valencia 2000 With Crop Load



Fig. 2-4. Effects of different induction levels and crop load treatments on the distribution of flowers by bud position along the shoot of 'Valencia', sweet orange trees during the 1999 and 2000 seasons. Data are presented as flowers per shoot.

Table 2-23. Results of stepwise multiple regression analyses relating the number of flowering buds per shoot (dependent variable) to temperature data in the form of the total number of hours in ranges of 5°C increments, average temperature, and crop load status (independent variables) for the combined data of the 1999 and the 2000 seasons

Dependent variable:	Cultivar: Hamlin					Cultivar: Valencia				
	Independent variables	Parameter estimate	F-Value	Pr > F	r ²	Independent variables	Parameter estimate	F-Value	Pr > F	r ²
Flowering buds Per shoot	Model		59.44	<.0001	0.79 **	Model		66.74	<.0001	0.88 **
	Intercept	0.71698	82.19	<.0001		Intercept	0.80466	62.86	<.0001	
	H below 15	0.00152	96.61	<.0001	0.64	Hrs. from 10 to 15	0.00187	99.45	<.0001	0.62
	Fruit	-0.40742	22.27	<.0001	0.14	H above 30	-0.00687	50.52	<.0001	0.22
						Fruit	-0.18159	8.55	0.0068	0.04

Table 2-24. Results of stepwise multiple regression analyses relating the number of flowers per flowering bud (dependent variable) to temperature data in the form of the total number of hours in ranges of 5°C increments, average temperature, and crop load status (independent variables) for the combined data of the 1999 and the 2000 seasons

Dependent variable	Cultivar: Hamlin					Cultivar: Valencia				
	Independent variables	Parameter estimate	F-Value	Pr > F	r ²	Independent variables	Parameter estimate	F-Value	Pr > F	r ²
Flowers per flowering bud	Model		3.42	0.0455	0.18 NS	Model		23.76	<.0001	0.62 **
	Intercept	2.6063				Intercept	1.00422			
	H from 10 to 15	0.00075	3.13	0.0869	0.10	H from 10 to 15	0.00291	41.12	<.0001	0.54
	Fruit	-0.29261	3.71	0.0632	0.08	Fruit	-0.38948	6.4	0.0171	0.08

Table 2-25. Results of stepwise multiple regression analyses relating the total number of flowers per shoot (dependent variable) to temperature data in the form of the total number of hours in ranges of 5°C increments, average temperature, and crop load status (independent variables) for the combined data of the 1999 and the 2000 seasons

Dependent variable	Cultivar: Hamlin					Cultivar: Valencia				
	Independent variables	Parameter estimate	F-Value	Pr > F	r ²	Independent variables	Parameter estimate	F-Value	Pr > F	r ²
Flowers per shoot	Model		30.44	<.0001	0.75 **	Model		49.23	<.0001	0.84 **
	Intercept	2.67469	26.96	<.0001		Intercept	1.65533	30.92	<.0001	
	H from 10 to 15	0.0067	55.21	<.0001	0.57	H from 10 to 15	0.00756	139.06	<.0001	0.59
	H above 30	-0.0079	2.64	0.1013	0.16	H from 25 to 30	-0.00413	35.5	<.0001	0.20
	Fruit	-1.35771	19.53	0.0001	0.02	Fruit	-0.54723	7.81	0.0093	0.04

Table 2-26. Summary of weather data for Lake Alfred, Florida during the 1998-1999 and 1999-2000 seasons

Date ^z	Trt. ^z	Avg. temp. (°C) ^x	Cumulative number of hours in each temperature range from 1 Oct.										
			Below 0	0 to 5	5 to 10	10 to 15	15 to 20	20 to 25	25 to 30	Above 30			
3 Nov 98	1	24.57	0	0	0	0	123	310	261	98			
17 Nov 98	2	21.12	0	0	1	31	230	434	331	102			
4 Dec 98	3	22.21	0	0	1	45	342	600	441	108			
18 Dec 98	4	19.52	0	1	18	91	456	695	503	108			
5 Jan 99	5	17.69	0	8	51	160	648	788	543	108			
19 Jan 99	6	15.39	7	28	100	228	748	854	568	108			
2 Feb 99	7	19.93	7	28	101	266	881	967	619	108			
16 Feb 99	8	18.91	7	32	124	304	1019	1046	673	108			
3 Mar 99	C ^w	15.94	7	44	177	384	1114	1116	698	108			
3 Nov 99	1	23.22	0	0	3	35	113	410	192	40			
18 Nov 99	2	18.88	0	0	14	83	277	498	241	40			
3 Dec 99	3	18.15	0	9	35	135	425	597	272	40			
16 Dec 99	4	18.59	0	9	48	180	566	685	297	40			
3 Jan 00	5	15.45	0	20	99	301	742	738	318	40			
18 Jan 00	6	17.63	0	20	131	383	878	807	359	40			
4 Feb 00	7	13.20	4	50	220	503	993	848	368	40			
3 Mar 00	C	17.45	4	59	283	666	1178	996	447	40			

^z Date correspond to the day in which the interruption of induction was performed in each set of branches (treatment dates)^y Identifier of induction level (treatment). - Treatment refers to the date in which floral induction was interrupted.^x Avg. temp. is the average temperature for the period comprised from the date of the previous induction treatment to the current. The first value corresponds to the average temperature from the 1 Oct.^w Control treatment was evaluated using weather data up to the 3 Mar.

CHAPTER 3
MODELING FLOWERING DATE OF SWEET ORANGE [*Citrus sinensis* (L.) Osbeck]
TREES IN CENTRAL FLORIDA BASED ON HISTORICAL WEATHER RECORDS

The flowering process in citrus consists of flower bud induction and differentiation processes preceding anthesis (flower opening). Both phenological processes (induction and differentiation) leading to bloom are primarily temperature dependent in subtropical climates (Cassin et al., 1969). Induction refers to the triggering of the flowering process by environmental factors resulting in a subsequent change in the pattern of the bud development, leading to flowering (Davies and Albrigo, 1994). Under subtropical conditions, citrus trees produce flowers primarily during the spring season following a winter rest period (García-Luis et al., 1995; Simanton, 1969). Vegetative buds are induced to be floral buds by low temperatures (Reuther et al., 1973). The first step towards the induction of flowers is a cessation of vegetative growth caused by low temperatures. The length of the low temperature period to which the buds are exposed directly influences timing and intensity of citrus flowering (Furr and Armstrong, 1956).

Differentiation involves the anatomical and morphological transition of a vegetative meristem to a floral meristem (Davenport, 1990). Vegetative and reproductive meristems are anatomically indistinguishable before differentiation starts, even if the flowering meristem is fully induced. In citrus, the differentiation of floral organs does not occur until the first stages of bud swelling and sprouting. The initiation of differentiation varies by citrus cultivar (Abbott, 1935). The morphological development of the flowers follows initiation of the flower primordia without interruption. The rate of

development depends on temperature and the number of flowers differentiating (Moss, 1973). Anthesis (flowering) occurs after induction followed by differentiation processes have been completed, usually when warmer temperature conditions exist. Not only time-to-bloom but also duration of peak anthesis is temperature dependent (Bellows and Morse, 1986; Lovatt et al., 1984).

A number of models have been developed to estimate time of flowering (Bellows and Morse, 1986; Ben Mechlia and Carroll, 1989; Lovatt et al., 1984). Each model uses temperature as the main input variable. These models cannot be extrapolated to Florida as they were developed using local weather information of Mediterranean type climates. Florida experiences a more irregular winter, causing the occurrence of peak flowering to vary from mid-February to mid-April. In addition, citrus trees in Florida often flower multiple times within one spring period. The objective was to analyze flowering records and compare them against historical weather data for central Florida to develop a model to predict the occurrence of peak anthesis, and to identify weather patterns that cause multiple blooms.

Materials and Methods

This study was based on the timing and duration of flowering data for Central Florida (around Lake Alfred) collected for the period between 1959 and 2000, and the historical weather data for the same period of time.

Duration of annual flowering periods and dates of peak anthesis (full bloom) for the entire period of time were obtained from published literature and direct field observations. The two most important published sources were the Florida Citrus Mutual newsletters and Simanton (1969). The Florida Citrus Mutual newsletters are weekly

publications that, among other information, provide descriptive information on the status of the crop by growing region. Every year, field-scouting observations report the status of the flowering season for locations throughout the Florida citrus production areas. Simanton (1969) also reported the occurrence and duration of citrus flowering for a period of 11 years (from 1959 until 1969) for central Florida. From these reports on flowering, it was possible to assemble a data set with information regarding timing and duration of the flowering season for the central Florida production area. Direct field observations, on dates of peak flowering of 'Valencia' groves for the period comprised from 1970 to 1984, were furnished by Dr. I. Stewart¹ (personal communication). From 1990 to date, direct flowering observations (dates of full bloom and duration of the flowering periods) were made by Dr. L. G. Albrigo² (personal communication).

The Florida Citrus Mutual newsletters described "extended bloom periods" for a number of years. In some years, two or more distinct blooms were described. An extended bloom period consists of groups of buds, branch sets (parts of trees), or different trees in a block flowering successively. This successive flowering (waves of bloom) provides the visual perception of the flowering process extending over a period of time, while each flower bud actually develops in the normal period of time. Although for all years a specific date of maximum anthesis was chosen, the years identified as having extended or distinct multiple flowering periods were not included with the years that had a single bloom for some analyses. Two data sets were thus formed: one set of all the

¹ Dr. I. Stewart is a retired horticulturist from the University of Florida's Citrus Research and Education Center (CREC), Lake Alfred, Fla.

² Dr. L. G. Albrigo is a horticulturist from the University of Florida's CREC, Lake Alfred, Fla.

data, and a second one containing data only for years with a single concentrated bloom period (Table 3-1). The later data set consisted of the remaining 20 years.

Historical weather data (from 1959 to 1998) were obtained primarily from National Oceanic Atmospheric Administration (NOAA) reports. The weather information included daily maximum and minimum temperatures, and rainfall from 1 Oct until 15 Apr. A weather generator (computer program) was used to estimate hourly weather data from daily maxima and minima for the same period of time. The weather generator assumes that the daily minimum temperature value occurs at 6:00 am, and fits a sine-wave curve for diurnal hours up to the maximum temperature (3:00 pm). It then estimates the evening hours by fitting a negative-logarithm curve until it reaches the next minimum temperature (at 6:00 am of the following day) (Ephrath et al., 1996). The output of the weather generator is hourly temperatures based on meteorological knowledge of daily weather patterns.

For 1998-2000, real-time weather measurements were obtained using the Florida Automated Weather Network (FAWN) system (FAWN, 2000). The FAWN system provides real time weather information in 15-minute intervals for locations throughout the state of Florida. The establishment of the weather information system provided accurate real-time temperature readings for Lake Alfred, Florida.

Analysis 1. Flowering Date as a Function of Average Monthly Temperatures

In the first analysis of the data, stepwise multiple regression analysis (SMRA) was used to relate flowering date against weather data for totality of the years comprised in this study. Flowering date was used as the dependent variable, whereas mean monthly temperatures and monthly rainfall (for October through April) were used as independent

variables. Mean monthly temperatures were obtained by averaging temperatures for all the hours within a given month.

Analysis 2. Flowering Date as a Function of Hours Accumulative Within Monthly Temperature Ranges

The objective of the second data analysis was to relate flowering date against the number of hours at different temperature intervals. In this analysis, temperature records (from November to March of all the years) were stratified into five temperature ranges (below 5, 5 to 12.8, 12.8 to 20, 20 to 30, and above 30°C) and the number of hours within each temperature range obtained. These ranges were then used as independent variables and flowering date used as dependent variables in SMRA.

Analysis 3. Days to Full Bloom as a Function of Stratified Degree-Day Analysis after Fulfillment of Induction Stimuli and Initiation of Differentiation.

The third analysis was performed using data from those years with only one identifiable flowering period. The objective of this analysis was to determine the degree-day requirements needed to complete flower development once differentiation has started. Since cool temperatures are needed for the induction of flower buds (Davenport, 1990; Furr and Armstrong) and warm temperatures for the differentiation processes (Lovatt et al., 1984; Bellows and Morse, 1986), it was desirable to establish a time separation between both processes. The main criterion used to separate induction from differentiation was that adequate growing conditions (warm period with at least 3 or 4 consecutive days with maximum temperatures above 23°C) was considered sufficient to trigger differentiation once an induction stimulus period had occurred (cool temperature accumulation). Based on recent observations, a number of warm spells were identified and based on field observations, and after some iterations, it was possible to determine the most likely warm period that triggered the onset of differentiation for each of the

analyzed years. Warm spells were characterized according to the previous inductive conditions that occurred, and the characteristics of the warm spell. A number of expert system rules were then developed that set the criteria needed for flower bud initiation (Table 3-2). It was then possible to determine the number of days to reach peak anthesis for each year as a function of accumulated degree-days for three temperature ranges (below 12.8, 12.8 to 20.8 and above 20.8°C). All analyses were done using multiple stepwise linear regression analysis procedures (SAS, NC State Version 7).

Analysis 4. Days to Full Bloom as a Function of Phenological Parameters After Fulfillment of Induction Stimuli and Initiation of Differentiation

Two final analyses were performed using data from years with only one identifiable flowering period. The objective of these analyses was to use floral differentiation data reported in the literature to obtain phenological parameters to analyze the historical data series. Phenological parameters for differentiation were obtained from research reported carried out under controlled temperature conditions (Moss, 1973). From the reported information, a weighed daily average temperature was obtained. As some differentiation temperatures in Florida normally exceed those reported in this study, additional differentiation information was obtained from research reported for field conditions of tropical environments (Cassin et al., 1969). Table 3-3 summarizes both controlled and field conditions that were used to establish the phenological parameters for floral development. An equation was developed that establishes the relationship between average daily temperature and days to full bloom (Fig. 3-1). This equation was developed under the assumption that the development of floral buds depends primarily on temperature and that flowers developed at a constant rate as indicated by the data of Moss (1973) and Cassin et al. (1969).

Determining differentiation time after fulfillment of induction stimuli and initiation of differentiation

As in the Analysis 3, it was necessary to separate the phenological processes of induction and differentiation. The main assumption used to separate induction from differentiation was that a warm period was considered sufficient to trigger the initiation of differentiation once enough inductive stimuli had occurred (cool temperature accumulation). Using the criterion previously described, induction and differentiation were separated after manual identification of warm periods.

The analysis of developmental temperatures was done on a daily basis. Hourly temperatures were averaged for a given day. Based on the daily average and the floral development equation, each daily average was assigned a fraction of differentiation time. If the daily average was assumed to produce bloom in an X number of days, then the corresponding fraction for each day was estimated to be X^{-1} . The developmental fractions for each day were added together for the total period from initiation to peak anthesis to obtain the total developmental fraction. This total developmental fraction was expressed in the percentage form by multiplying it by 100. Theoretically, the final developmental fraction should be close to 100% development.

Determining date of initiation of differentiation based on the reported date for peak anthesis

The objective of this approach was to determine if the equation for floral development could be used to predict the date of initiation of differentiation, starting on the date of peak anthesis and going back. The equation for flower development equation was used to assign a development fraction to the totality of the days leading to peak anthesis. The date of initiation of differentiation was estimated by accumulating fractions up to the date of peak anthesis. The date when the total summation equaled 100% was

estimated to be the theoretical initiation time. A characterization of the induction stimulus occurring before initiation of differentiation was obtained.

Results

The several approaches listed were followed to obtain mathematical equations that relate variability in climate to the variability in occurrence of citrus flowering

Analysis 1. Flowering Date as a Function of Average Monthly Temperatures

Stepwise multiple regression analysis (SMRA) was used to relate bloom date (dependent variable) to mean monthly temperatures or monthly rainfall (independent variables) for all of the 40 years of data. The average temperatures of December, January, and February were significantly associated with date of flowering (Table 3-4). The stepwise entry of mean temperatures for these three months was December, February followed by January. The mean temperatures of October, November, and March were not associated with date of flowering by this statistical method. No rainfall data for any month was associated with date of flowering. Warmer temperatures in December and January were associated with later bloom dates, while warmer temperatures in February were associated with earlier bloom dates. The resulting equation accounted for 43.1% of the variability.

Analysis 2. Flowering Date as a Function of Hours Accumulated Within Monthly Temperature Ranges

A second SMRA was performed using hourly temperature records (November to March of each year) after they were stratified into five temperature ranges (below 5, 5 to 12.8, 12.8 to 20, 20 to 30, and above 30°C) and the monthly number of hours within each temperature range obtained. These monthly hours (independent variable) were related to days to flowering (dependent variables) (Table 3-5). Low temperatures for the period

comprising much of the induction period (November and December) were significantly associated with bloom date, but temperatures below 5°C that occurred in November were related to delayed bloom, while these low temperatures if they occurred in December were associated with earlier bloom. Both 5 to 12.8°C in February and 20 to 30°C in January were related to later flowering dates and only temperatures above 30°C in January were associated with earlier flowering.

Analysis 3. Days to Full Bloom as a Function of Stratified Degree-day Analysis after Fulfillment of Induction Stimuli and Initiation of Differentiation.

SMRA was also used to determine the temperature requirements needed to complete the days to peak anthesis after an induction stimuli and the initiation of differentiation. Having identified the most likely warm period, degree-days were obtained for different temperature ranges in the whole spectrum of temperatures occurring. The SMRA performed used days to peak anthesis as the dependent variable and degree-days ((hourly temperature - threshold)×24⁻¹) for different temperature ranges as independent variables found that degree-days for temperatures below 12.8°C and from 12.8 to 20.8°C were associated with extended differentiation time (later bloom dates), whereas hours at temperature above 20.8°C reduced the time to bloom (Table 3-6). Table 3-7 presents a comparison of observed vs. predicted differentiation time based on stratified degree-day analysis.

A mathematical procedure was developed to estimate daily accumulation of floral development (percent) from 0 at initiation to 100% at anthesis. This procedure assumes floral development to be constant throughout the differentiation cycle. If in a given year, n days elapsed from start of differentiation to full bloom, it was assumed that n⁻¹ of the development occurred each day. According to the results of this analysis, three degree-

day variables significantly affect time-to-flower: degree-days below 12.8, degree-days from 12.8 to 20.8, and degree-days above 20.8. If in a given year there were X, Y, and Z number of degree-days on each category, it was possible to divide by n to obtain the daily contribution of each variable (X, Y, and Z) to the daily accumulation. In order to generate the data points needed to obtain an equation that would accumulate floral development, four more "artificial" variables (A, B, C, and D) were created to form the equation of $n^{-1} = (X \times n^{-1})A + (Y \times n^{-1})B + (Z \times n^{-1})C + D$. From this equation a new set of data points was generated from each year. A new regression analysis was made to estimate A, B, C, and D parameters. Based on this equation, it was possible to express flower development in a percentage basis from 0 at initiation to 100% at flower opening (Table 3-8). The equation details are the following:

$$PFD = 0.0183 + (0.0000407 \times X') + (0.000195 \times Y') - (0.000228 \times Z')$$

Where:

PFD = Percent of Floral Development

X' = Cumulative degree-hours below 12.8°C,

Y' = Cumulative degree-hours above 12.8°C, and

Z' = Cumulative degree-hours above 20.8°C.

*

Analysis 4. Days to Full Bloom as a Function of Phenological Parameters after Fulfillment of Induction Stimuli and Initiation of Differentiation.

Determining differentiation time after fulfillment of induction stimuli and initiation of differentiation

Based on average daily values for floral development, an equation was established that estimates the total amount of days (n) that constant temperatures would cause

flowering to occur. Therefore, each average daily temperature was considered to cause (n^{-1}) of the total differentiation time. The equation details are the following (Fig. 3-1):

$$DF = (-0.0664 x^3 + 3.8491 x^2 - 76.314 x + 575.56)^{-1} \quad (R^2=0.9992),$$

Where:

DF = Developmental fraction for each average daily temperature

x = Average daily temperature for any given day.

Having identified the warm period triggering the initiation of differentiation and based on the total fraction for each day leading to anthesis, a total developmental fraction for the whole period (from initiation to anthesis) was estimated. The total developmental fractions for all the years with one single identifiable flowering period are presented in Table 3-9.

Determining date of initiation of differentiation based on the reported date for peak anthesis

Based on the observed date of peak anthesis and the equation for flower development as a function of phenological parameters, development fractions were estimated for the totality of days leading to peak anthesis. The date when the summation of the partial developmental fractions equaled 100% was estimated to be the initial onset of differentiation. A list of the initiation dates based on the warm period method is presented in Table 3-10 along with a characterization of the inductive stimulus occurring before the initiation of differentiation.

Discussion

This study was aimed at understanding the effects of temperature and rainfall on citrus flowering in Florida. As in other studies (Bellows and Morse, 1986; Ben Mechlia and Carroll, 1989; Lovatt et al., 1984), temperature was more important than rainfall in

affecting the timing of citrus flowering. Several analyses were made to develop statistical models that estimate the expected date of citrus flowering based on temperature and rainfall data. As rainfall did not enter the equations as a significant factor in any of the analyses, it may be considered that rainfall is not a consistent factor in floral induction for subtropical climates as it is in the tropics.

The statistical model, that related bloom date to average mean monthly temperatures for the fall and winter periods, associated warmer temperatures in December and January with a later bloom date (Table 3-4). Warm December temperatures could delay the induction process and cause differentiation to start later. Usually, differentiation of citrus flower buds occurs by mid-January (Abbott, 1935) and warmer January temperatures might be expected to reduce the time to bloom. It is possible that low temperatures in January still delay initiation of differentiation. Higher temperatures in February were associated with early flowering dates and warm temperatures within the physiologically moderate range would be expected to hasten the differentiation and bud swell processes and therefore the time to bloom. This type of model does not allow predicting the date of bloom as at least four physiological processes occur over the fall, winter, and spring time period (flower bud induction, initiation of differentiation, differentiation and bud swelling). The year-to-year variation in temperatures and therefore in the date at which the differentiation process begins makes it impossible to predict the date of flowering from mean monthly temperatures. Since multiple blooms can occur in Florida during the spring, predicting bloom date is even more complicated.

Relating flowering date to the number of accumulated monthly hours in the different temperature ranges has an advantage if there is an expected biological response to a minimum threshold temperature (12.8°C for vegetative growth of citrus (Girton, 1927)) and more rapid development as temperature increases above this temperature toward some optimum. However, the equation developed had several deficiencies in explaining the associated temperatures on a physiological basis. Temperatures below 5°C in November and December were related to bloom date, but November's temperatures increased days to bloom and December's decreased days to bloom. Further, these temperatures are presumed to be below the physiological range for citrus flower bud induction. Temperatures below 5°C failed to induce floral buds in vitro (García-Luis et al., 1992). Temperatures between 5 and 12.8°C in February (third variable) would be expected to delay bloom date, but temperatures of 20 to 30°C in January would be expected to shorten the time to bloom by accelerating differentiation. Even if these temperatures occurred early in January, they would be expected to cause initiation of differentiation (Davenport, 1990) if it had not already occurred. Accumulated hours above 30°C in January did relate to earlier bloom dates. Temperatures above 30°C decreasing days to bloom seems reasonable but the association of 20 to 30°C temperatures for this same period with increased number of days to bloom is not logical. However, the statistical procedure of multiple regression indicates an association of 20 to 30°C hours with later blooms, it does not prove a cause and effect relationship. This model again falls short of relating temperatures with flowering date in a clear physiological way. The model does not distinguish between physiological events and does not account for all of the temperature effects of all time periods over which the

flower bud development processes occur. SMRA is only able to relate variations of one or more factors to variations in another factor (dependent). If there is little variation in temperatures for a given time period, SMRA will not suggest a relationship, even though that temperature range at that time may significantly stimulate induction or differentiation.

The third model presented here avoids complications related to modeling multiple physiological events by relating only temperatures that occur for the time period from initiation of differentiation to peak bloom (Table 3-8). There was a very good fit, accounting for 94% of the variability in bloom dates. Degree-days (DD) accumulated below 12.8°C (negative DD) were related to later bloom, but DD in the 12.8 to 20.8°C range were also associated with later bloom dates. Only DD in the + 20.8°C temperature range were associated with earlier bloom dates. Although flower bud development in a Mediterranean climate may require four months to progress from initiation of differentiation to full bloom (Lord and Eckard, 1985), it does occur at these typically lower late winter and spring temperatures. Almost all of this time period is at temperatures below 20.8°C. This model accounts for all of the temperatures that occur from initiation of differentiation to bloom, but it does not relate these temperatures in an expected physiological manner, at least at this point in our understanding of the process of flower bud development. It is possible that DD calculated for smaller temperature increments would solve some of the problems mentioned above, but preliminary attempts did not result in either a better fit of DD to time to bloom nor in an physiologically sound equation (not shown).

The fourth analysis represents the first attempt to use phenological parameters to explain and predict citrus flowering. These phenological parameters (represented in a floral development equation) were based on published reports of the effects of temperature on flowering time. The most important assumption made to develop this equation was that temperature was the single most important factor influencing floral development. This broad assumption may limit the applicability of the floral development equation (regardless of its good fit). Nevertheless, based on this equation, it was possible to obtain an average forecasting for the floral development equation of 97.26% and a difference in days from the initiation time based on a warm period of 4.3 days. There was, however, considerable variability of 14.5% and 11 d, respectively. The input of average daily temperatures into the floral development equation provided the best forecasting results. Average daily temperatures do not take into account fluctuations in daily temperature. Attempts to assign a developmental fraction to each hourly temperature increased the error and variability considerably (not shown).

The use of phenological parameters, based on research under controlled temperature conditions, is the best alternative to statistically based models from local weather parameters. The use of phenological parameters should be able to take into consideration all the temperatures that occur from initiation of differentiation to anthesis.

Models that are based exclusively on environmental factors but without a physiological basis may not be easily extrapolated to other production regions. Florida's irregular winters with intermittent warm periods probably makes the weather based models unsuitable to be exported into Mediterranean environments. In addition, differentiation time in Florida does not have enough hours in the high temperature range

(above 25°C), which would allow the models to be used in tropical environments. Due to the inherent data collection limitations of using historical data, this study is not able to address the effects of other factors on flowering such as crop load from the current and previous year, introduction of irrigation, effect of freezes, or other possible factors.

The use of phenological parameters is the logical alternative to statistical models based on local weather information. However, further research is needed to obtain better phenological parameters throughout the entire flowering cycle. It is therefore necessary to understand the temperature dynamics causing flower bud induction and differentiation before adequate phenological models can be developed. In addition, understanding the dynamics of induction temperatures and warm periods throughout the wintertime should also explain the occurrence of multiple and extended flowering periods. The modeling attempt that best fit bloom date to climatic data dealt only with winters without multiple or extended bloom periods. Further modeling using the warm period principle as an initiator of a bloom and applying it to multiple bloom years is needed to fit the Florida citrus bloom situation.

Conclusions

Four temperature-dependent models aimed at the understanding and prediction of citrus flowering were developed. The statistical models that estimate flowering date as a function of average monthly temperatures, or hours within monthly temperature ranges, or stratified degree-day analyses yielded reasonable results. However, as local weather data were used in their development, these models may be used in Florida, but may not be extrapolated to other production regions (e.g. Mediterranean type or tropical climates). Statistical models based on local weather factors tend to emphasize the climatic features

of the area for which they were developed. Although statistical models may have good fit, they also have little universality.

An ideal model should use physiological parameters that emphasize physiological response to environmental conditions. The fourth model that uses phenological parameters use the logical alternative to statistical models based on local weather information. However the phenological parameters hereby used need to be improved. Further research is therefore needed to obtain better phenological parameters throughout the entire flowering cycle.

Table 3-1. Data records for 20 years of flowering dates for oranges in Central Florida in which a single bloom period was detected. Sources: Florida Citrus Mutual newsletters, Simanton (1969), and personal observations.

Year	Days to full bloom ^z	Reported date of peak anthesis	Average temperature during flower development (°C)
1960-1961	70	7-Mar	16.20
1962-1963	64	19-Feb	14.58
1965-1966	80	21-Mar	14.99
1966-1967	84	25-Mar	16.97
1968-1969	71	22-Mar	14.27
1970-1971	66	11-Mar	16.60
1972-1973	76	16-Mar	16.31
1975-1976	69	2-Mar	14.44
1976-1977	87	7-Mar	12.58
1977-1978	86	10-Mar	12.65
1978-1979	68	16-Mar	14.53
1979-1980	70	19-Mar	15.63
1981-1982	55	15-Feb	17.27
1983-1984	58	24-Feb	14.30
1984-1985	84	8-Mar	15.71
1985-1986	69	15-Mar	15.13
1987-1988	88	17-Mar	14.93
1988-1989	54	12-Feb	18.02
1990-1991	72	26-Feb	18.52
1999-2000	70	11-Mar	16.88

^z. Days from initiation of differentiation (based on a warm period) to full bloom

Table 3-2. Induction monitoring expert system rule database set for the determination of induction status

Induction level	Inductive conditions ^z	Description of induction level	Weather conditions needed for initiation
Low	0-700 h below 20°C	Induction stimuli is not sufficient to cause economic crop levels of floral organ differentiation	No economically significant initiation is expected
Moderate	700-1000 h below 20°C	Induction stimuli is sufficient that economic levels of buds would be expected to initiate and develop flowers if warm conditions occur	700-850 h below 20°C. Twelve days with maximum temperatures above 25°C with no more than 2 intervening days below 20°C 850-1000 h below 20°C. Seven days with maximum temperatures above 20°C, with no more than three intervening days below 20°C
High	1000-1200 h below 20°C	Warm conditions with sufficient soil moisture will initiate enough differentiation that no additional flowering is likely	7 days with maximum temperatures above 20°C with no more than two intervening days below 20°C
Excessive	Above 1200 h below 20°C	If initiation has not occurred, excessive flowering would be likely to occur	5 consecutive days with maximum temperatures above 20°C with no more than 4 intervening days below 20°C

^z Inductive conditions refer to the number of hours below 20°C from 1 Oct. to the current date

Table 3-3. Phenological parameters from reported data in the literature.

Temperature Conditions	Average Daily Temperature	Number of Days Elapsed from Initiation to Full Bloom	Source
8 hours at 15°C and 16 hours at 10°C	11.67	104	Moss (1973)
8 hours at 18°C and 16 hours at 13°C	14.67	74	
8 hours at 21°C and 16 hours at 16°C	17.67	64	
8 hours at 24°C and 16 hours at 19°C	20.67	56	
From Cassin et al (1969)	26.33	23.333	

Average Monthly Temperature	Time Elapsed from Initiation to Full Bloom	Source
27.2°C	23 days	Cassin, et al, (1969)
25.9°C	24 days	
25.9°C	23 days	
Averages 26.33°C	23.33 days	

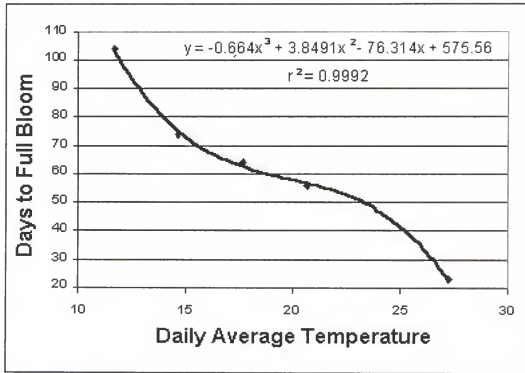


Fig. 3-2. Floral development equation. The association of average daily temperatures with days to full bloom based on reported differentiation time. If the average daily temperature was assumed to cause flowering to occur in n days then the corresponding development fraction for that day was n^{-1} .

Table 3-4. The association of date of flowering of orange trees with significant monthly mean temperatures by stepwise multiple regression analysis in Florida for 40 years (1959-1998).

Dependent Variable	Independent Variable	Parameter Estimate	F-Value	$r^2 = 0.4311$
Date of flowering	Intercept	48.57043	9.36	
	December	2.06087	7.85	
	January	1.4139	5.43	
	February	-1.7957	7.58	

Table 3-5. The association of date of flowering with significant monthly hours of stratified temperatures for oranges in Central Florida for a 40-year period (1959-1998). Stepwise multiple regression analysis using temperature records (November to March of each year) stratified into five temperature ranges (below 5, 5 to 12.8, 12.8 to 20, 20 to 30, and above 30°C) was performed.

Dependent Variable	Independent Variable	Parameter Estimate	F-Value	$r^2 = 0.6619$
Date of flowering	Intercept	61.20734	178.62	
	Nov below 5	0.29258	13.79	
	Dec below 5	-0.16455	22.33	
	Feb5-12.8	0.05247	9.36	
	Jan 20-30	0.04632	9.60	
	Jan above 30	1.81028	5.65	

Table 3-6. Stepwise multiple regression analysis of days to peak anthesis to degree-days ((hourly temperature - threshold) $\times 24^{-1}$) for different temperature ranges. The best degree-day ranges for this analysis to maximize accounting for variability were: below 12.8°C, from 12.8 to 20.8°C, and above 20.8°C. Initiation of differentiation was determined manually by examining the occurrence of warm periods after mid-December.

Dependent Variable	Independent Variable	Parameter Estimate	F-Value	$r^2 = 0.9439$
Days to peak anthesis	Intercept	6.40436	2.05	
	DD Below 12.8 ^z	0.13267	123.98	
	DD 12.8 to 20.8 ^y	0.27848	106.64	
	DD Above 20.8 ^x	-0.35277	37.61	

^z Cumulative degree-days below 12.8 from initiation to the date of peak anthesis

^y Cumulative degree-days from 12.8 to 20.8 from initiation to the date of peak anthesis

^x Cumulative degree-days above 20.8 from initiation to the date of peak anthesis (whenever the temperature is above 20.8 degree-hours from 12.8 to 20.8 also accumulate the maximum value of 8).

Table 3-7. Comparison of observed vs. predicted days to full bloom based on degree-day analysis.

Year	Days to Full Bloom ^z	Days to Full Bloom (Predicted) ^y	Difference ^x
1960-1961	70	67.53	-2.5
1962-1963	64	63.31	-0.7
1965-1966	80	81.34	1.3
1966-1967	84	84.64	0.6
1968-1969	71	65.04	-6.0
1970-1971	66	64.41	-1.6
1972-1973	76	70.95	-5.0
1975-1976	69	70.00	1.0
1976-1977	87	92.55	5.6
1977-1978	86	85.95	-0.1
1978-1979	68	69.01	1.0
1979-1980	70	70.92	0.9
1981-1982	55	59.99	5.0
1983-1984	58	58.29	0.3
1984-1985	84	80.64	-3.4
1985-1986	69	72.13	3.1
1987-1988	88	83.65	-4.4
1988-1989	54	53.55	-0.5
1990-1991	72	76.33	4.3
1999-2000	70	71.83	1.8

^z Number of days to full bloom from the date of initiation (determined by the occurrence of a warm period) to the date of peak anthesis.

^y Number of days to full bloom from the date of initiation (determined by the occurrence of a warm period) to the date of

^x Difference in days from the two methods

Table 3-8. Transformation of equation from Table 3-6 into percent of floral development.
 Percent of floral development = $AX' + BY' + CZ' + D$

Dependent variable	Independent variable	Parameter estimate	$r^2 = 0.9439$
Percent of floral development	Intercept	0.0183	
	Cumulative degree-hours below 12.8°C (X')	0.0000407008	
	Cumulative degree-hours above 12.8°C (Y')	0.000194996	
	Cumulative degree-hours above 20.8°C (Z') [‡]	-0.000227703	

[‡] Whenever the temperature is above 20.8°C degree-hours above 12.8°C also accumulates the maximum value of 8

Table 3-9. Total percentage of floral development from initiation to peak anthesis based on the floral development equation.

Year	Total Days from Initiation to Full Bloom ^z	Total Developmental Fraction (%)
1960-1961	70	101.39
1962-1963	64	80.47
1965-1966	80	105.48
1966-1967	84	125.55
1968-1969	71	92.49
1970-1971	66	93.23
1972-1973	76	111.77
1975-1976	69	83.29
1976-1977	87	91.54
1977-1978	86	93.56
1978-1979	68	87.37
1979-1980	70	94.44
1981-1982	55	79.80
1983-1984	58	69.74
1984-1985	84	116.02
1985-1986	69	105.22
1987-1988	88	111.19
1988-1989	54	83.43
1990-1991	72	115.10
1999-2000	70	104.23
Average		97.26
S.D.		14.50

^z Number of days from the date of initiation (determined based on the floral development equation) to the date of peak anthesis using the floral development equation.

^y Percent of developmental fraction for the same period of time using the floral development equation.

Table 3-10. Comparison of the estimated initiation time based on the floral development equation and after a warm period was considered to have initiated differentiation. A comparison of total inductive hours before each alternative initiation time is presented.

Year	Estimated Initiation Date Based on Equation For Floral Development	Estimated Initiation Date Based on Warm Period	Difference (Days) ^z	Number of Hours Below 20°C During Induction. Initiation Date Based on Equation for Floral Development ^y	Number of Hours Below 20°C During Induction. Initiation Date Based on Warm Period ^x
1960-1961	29 Dec.	27 Dec.	-2.0	1056	1020
1962-1963	27 Nov.	17 Dec.	20.0	690	1129
1965-1966	4 Jan.	1 Jan.	-3.0	1278	1234
1966-1967	18 Jan.	1 Jan.	-17.0	1591	1316
1968-1969	1 Jan.	10 Jan.	9.0	1274	1474
1970-1971	27 Dec.	4 Jan.	8.0	1058	1240
1972-1973	6 Jan.	30 Dec.	-7.0	1121	1037
1975-1976	10 Dec.	25 Dec.	15.0	658	931
1976-1977	5 Dec.	10 Dec.	5.0	927	1013
1977-1978	9 Dec.	14 Dec.	5.0	832	936
1978-1979	27 Dec.	7 Jan.	11.0	907	1092
1979-1980	4 Jan.	10 Jan.	6.0	1171	1304
1981-1982	2 Dec.	23 Dec.	21.0	664	1093
1983-1984	6 Dec.	29 Dec.	23.0	631	1052
1984-1985	22 Dec.	14 Dec.	-8.0	961	842
1985-1986	3 Jan.	6 Jan.	3.0	937	978
1987-1988	27 Dec.	21 Dec.	-6.0	1155	1084
1988-1989	7 Dec.	21 Dec.	14.0	768	1047
1990-1991	25 Dec.	16 Dec.	-9.0	808	712
1999-2000	3 Jan.	1 Jan.	-2.0	1160	1131
Average			4.3	982.4	1083.3

^z Difference in days comparing both methods to determine initiation of differentiation.

^y Number of hours below 20°C from 1 Oct. up to the estimated date of initiation of differentiation based on the flower development equation.

^x Number of hours below 20°C from 1 Oct. up to the estimated date of initiation of differentiation based on the warm period method.

CHAPTER 4

INTEGRATION CHAPTER. DECISION SUPPORT SYSTEM FOR THE PREDICTION, AND MANAGEMENT OF THE FLOWERING PROCESS OF SWEET ORANGE TREES [*Citrus sinensis* (L.) Osbeck] UNDER FLORIDA CONDITIONS

A primary objective of phenology studies has been to observe developmental events. Based on data from physiological studies, it has been possible to develop models capable of explaining the occurrence of phenological events (Schwartz et al., 1997). A desirable phenological model should not only explain historic information accurately, but should also have good predictive ability (Ben Mechlia and Carroll, 1989a; Ben Mechlia and Carroll, 1989b). Modeling has become a useful tool in integrated studies of crop-weather relationships. As crop growth models constitute a convenient way of synthesizing a variety of information into a single package (Ben Mechlia and Carroll, 1989b), they are now being used to inform growers of the stage of development of their crop during the growing season. In turn, this information can be used to efficiently schedule farm operations (Schwartz et al., 1997).

An Expert System (ES) is a computer program aimed at mimicking the human expert's reasoning process and knowledge in specific fields. ES apply specific knowledge and thought processes through structured methods called artificial intelligence (Bentham, 1998; Jackson, 1990; Waterman, 1986). In agriculture, most ES manipulate knowledge, search for connections and relations between different patterns, and provide diagnostics or recommendations to nonspecialists (Nolasco-Bethencour, 1998).

A Decision Support System (DSS), in comparison to an expert system, is a computer program, which solves problems spanning over several knowledge or problem

areas. This is accomplished by combining information, knowledge, and human expertise, through the integration of expert systems, rule-sets, site-specific data, and any other combination of software and/or information, which can aid in the decision-making process through interactions with the end-user (Bentham, 1998).

An ideal DSS for citrus should have a phenology-based component to match production practices to the actual needs of field trees. From the point of view of fruit production, each production season begins with the induction and the development of floral buds. A number of temperature-based phenological models have been developed to explain and predict citrus flowering (Bellows and Morse, 1986; Ben Mechlia and Carroll, 1989b; Lovatt et al., 1984). These models are statistical relations based on local weather factors. A problem with statistical models is that as more climatic factors are used in the regression equation, the more strongly the climatic features of the area for which they were developed are emphasized. These models were developed for a Mediterranean type climate, which makes them generally unsuited for other production regions (i.e. tropical or subtropical humid regions) (Chapter 3).

This DSS consists of an expert system for the monitoring of flower bud induction and differentiation processes that is based on a weather monitoring system. This DSS is linked to expert-system rules for practical recommendations to growers for the management of citrus flowering. This DSS is being developed as part of the Decision Information Systems for Citrus (DISC). This part of the DSS consists of expert systems that run based on a weather monitoring system for 1) the monitoring of flower bud induction, and 2) the prediction of differentiation and flowering processes. The incorporation into this expert system of practical recommendations for the management

of citrus flowering is also described. Expert system rules for management of citrus flowering were developed and reviewed by domain experts and will be tested during the 2001-2002 cycles in central Florida.

Materials and Methods

Overall Framework of the DISC-DSS

The DISC-DSS was programmed in Java as this programming language provided a flexible tool for joining a wide array of components, and it can be executed under a number of platforms. The DISC-DSS is composed of three main elements: a calendar component, a GPS-GIS component, and the phenological component. The calendar component was incorporated as a standard Java component to provide the user with date specific recommendations, as well as record keeping capabilities. The GPS-GIS component was incorporated for the spatial integration of citrus groves. This was needed as the majority of citrus groves in Florida are managed under different production blocks. The GPS-GIS component provided the mapping system for production blocks. The phenological component was programmed as flower bud induction and differentiation phenological modules. Both modules consist of Java programs that contain a weather data acquisition system and a set of instructions for the monitoring and management of the phenological response. Both modules are linked to Expert Systems for their onset, as well as to provide recommendations for the management of the phenological status.

Induction Monitoring Expert System

The Induction Monitoring Expert System (IMES) consists of three basic components: a set of rule-based parameters to monitor floral bud induction based on weather data, and a set of expert system rules to determine induction status, and practical

recommendations for its management. All these components are linked to a weather data acquisition system to provide recommendations on a real-time basis.

The parameters for the monitoring of floral induction status were determined with field experiments using mature field trees exposed to natural low temperature inductive conditions, analysis of historical data for central Florida, and previously collected information (Albrigo, 1998b).

Field experiments using mature field trees, exposed to low temperature inductive conditions, were used to determine the parameters for the Induction Monitoring Expert System (IMES). Weather information was obtained using real-time weather measurements obtained from the Florida Automated Weather Network (FAWN) system (FAWN, 2000). The flowering phenological response was recorded, and compared against weather information using stepwise multiple regression analysis procedures (SAS, NC State Version 7) (Chapter 2 for details).

Induction conditions were characterized based on the analyses of historical flowering data for Central Florida (around Lake Alfred, Florida) for the period from 1959 to 2000, and the historical weather data for the same period of time (Chapter 3 for details). Duration of annual flowering periods and dates of peak flowering were assembled. As some years were reported to have a concentrated flowering period while for others "extended flowering periods" were reported. Induction was characterized using exclusively a subset of the data (20 yr.) that consisted of years with a single concentrated flowering period. Historical weather data (from 1959 to 1998) were obtained and generated from NOAA reports. For 1998-2000, real-time weather measurements were obtained using the FAWN system.

The parameters to determine the status of floral bud induction (table 4-1) were established based on the existing inductive conditions before the onset of differentiation (initiation) determined by the warm period or the theoretical onset of differentiation based on the floral developmental equation and the reported date of peak anthesis (next section on Transition from Induction to differentiation models).

Expert system rules for the management of citrus flowering were developed by means of a review of published information (Chapter 1). Published information was complemented with formal and informal interviews with domain experts and cooperating growers. All the rules were finally reviewed by domain experts and programmed using Java programming language.

Transition from Induction to Differentiation Models

A set of rules was developed to separate the induction and differentiation processes (see table 4-1). These rules are based on the principle that citrus buds do not enter into deep endodormant stages. Therefore, after sufficient inductive conditions have occurred, the occurrence of adequate conditions for shoot growth will trigger bud sprouting. During the winter time, citrus buds are considered to be on ecodormant stage. Ecodormant buds are able to sprout if favorable growth conditions release the stress caused by low temperatures (Lang et al., 1987). Therefore, citrus buds may be environmentally triggered when adequate growing conditions exist. For purposes of modeling, the onset of differentiation (initiation) of flower buds was expected to occur when adequate conditions for growth (warm conditions and sufficient soil moisture) were present. A set of rules was developed based on field observations and examination of historical weather records (of years with one identifiable flowering period) to determine the growing conditions needed to sprout flowering buds (Chapter 3 for details).

Models for Floral Organ Differentiation – Time to Bloom Models

Two time-to-bloom models were developed based on the analysis of timing and duration of flowering data for Central Florida (around Lake Alfred, Florida) collected for the period between 1959 and 2000, and the historical weather data for the same period of time (Above and Chapter 3 for details in methodology). Models were developed using years with a single concentrated flowering period and the historical weather data (regenerated and real-time weather measurements).

Onset of differentiation was determined when a warm period and adequate induction levels allowed growth to occur (see Table 4-1). The number of days to reach peak anthesis was used as the dependent variable and compared against the accumulated degree-days for three temperature ranges after the initiation of differentiation (below 12.8, 12.8 to 20.8 and above 20.8°C) using procedures for stepwise multiple linear regression analysis (SAS, NC State Version 7). The model estimates percent of floral development as a function of stratified degree-day analysis (Chapter 3 for details). The equation details are the following:

$$PFD = 0.0183 + 0.0000407 \times A + 0.000195 \times B - 0.000228 \times C$$

Where:

PFD = Percent of Floral Development

A = Cumulative degree-hours below 12.8°C ,

B = Cumulative degree-hours above 12.8°C, and

C = Cumulative degree-hours above 20.8°C.

A second analysis used floral differentiation data reported in the literature to obtain phenological parameters to analyze the historical data series (Cassin et al., 1969; Moss, 1973). From published information, an equation was developed that relates

weighed daily average temperature and days to full bloom (Chapter 3 for details). The model uses average daily temperature to estimate developmental fractions for floral development for each average daily temperature. Cumulatively developmental fractions estimate accumulation of floral development (percent) from 0 at initiation to 100% at flower opening. The equation details are the following:

$$DF = (-0.0664 x^3 + 3.8491 x^2 - 76.314 x + 575.56)^{-1} \quad R^2 = 0.9992,$$

Where:

DF = Developmental fraction for each average daily temperature

X = Average daily temperature for any given day.

Results

Overall Framework of the DISC-DSS

An overall framework was developed in Java and encompasses an input-output area for phenology and production practices, a calendar based scheduling system, and a GPS-GIS system for block specific functions. In the main screen, the input-output area displays three zones under the time bar: phenology stages, recommended production practices, and actual practices applied. A task bar is above this area to allow input changes and pull-ups of linked programs. The calendar based scheduling system (positioned to the upper right) activates status boxes or points to current phenology stage and recommended practices for a highlighted block or blocks in a GPS-GIS map (located in the lower right). Specific blocks or groups of blocks can be highlighted in relation to cultivar, rootstock, irrigation zones, need for a spray or any other function.

Induction Monitoring Expert System

An expert system was developed that determines the floral induction status of the tree on a real-time basis, and provides growers with practical recommendations for the management of the flowering period. The Expert System for Induction Monitoring determines induction level based on a set of expert system rules for the determination of floral induction status, and a weather data acquisition system. The weather data need to be collected on a real-time basis for a number of locations located in the citrus production zones of Florida. An 8-day weather prediction is incorporated into the induction monitoring system. Expert system rules are triggered based on the weather information induction model (both historical and forecasted), and crop status (tree age, and cultivar primarily).

Starting 1 Oct. the program starts the accumulation of number of hours below 20°C and continues during the entire induction period. During this period, three different floral induction statuses are determined by monitoring local weather from Oct to the current date of each season. These statuses are low, moderate, or high induction levels (Table 4-1). Low induction levels refer to the period of time at the beginning of the winter period when the induction stimuli is not sufficient to cause economic crop levels of floral organ differentiation. Moderate induction levels refer to the period of time when induction is sufficient that economic levels of buds would be expected to initiate and develop flowers if a warm conditions occurs. At the low end of moderate induction levels and without induction enhancement, the occurrence of multiple cool conditions interspersed with warm conditions and sufficient soil moisture conditions can still lead to the occurrence of multiple or extended flowering periods. High induction levels refer to the period of time when enough induction stimuli has occurred that warm conditions with

sufficient soil moisture will initiate enough differentiation that no additional flowering is likely.

Based on induction levels the IMES provides a series of recommendations to growers for the management of the flowering period. Expert system rules developed for the four different management strategies and incorporated into the rule for the flower bud induction expert system include: 1) strategy to delay the initiation of differentiation for the management of secondary or extended flowering periods; 2) enhancement of flower bud induction; 3) reduction of induction levels; and 4) management of secondary or extended flowering.

1) Strategy to delay the initiation of differentiation for the management of secondary or extended flowering periods. During low or early moderate induction levels, the presence of conditions for the initiation of flowers will likely cause the sprouting of floral buds. The subsequent occurrence of induction and differentiation conditions after additional cool periods will cause more floral buds to be induced and develop causing extended or multiple flowering periods. The expert system monitors induction level and recommends the reduction of soil water availability by withholding irrigation during low to the early moderate induction levels (Tables 4-2 and 4-3). Low soil moisture availability will prevent growth, and it may add to the inductive conditions of low temperature.

Citrus production areas in Florida have different soil water holding capacities. Therefore, the amount of time that irrigation needs to be withheld to reduce water availability varies according to the location of the trees. For the management of citrus flowering, the expert system recommends to withdraw irrigation sometime before the

trigger from low to moderate induction levels depending on soil water retention characteristics. In deep sands, water availability may be reduced after a period of 2 to 3 weeks. If the groves are in soils with low soil water retention (i.e. shallow depth soils or in bedded groves), water availability may be reduced in 3 to 4 days.

Irrigation withdrawal should be approached carefully in certain cultivars such as 'Hamlin' or 'Pineapple' as it may cause fruit drop. 'Valencia' trees, with late maturing fruit, can withstand more water stress than other cultivars.

2) Enhancement of flower bud induction. Alternate bearing (AB) is the tendency of fruit trees to produce a heavy crop (ON-years) one-year followed by a very light crop (OFF-year) the following year. Low crop productivity during the OFF-year is primarily due to lack of flower formation during the ON-year (Goldschmidt and Golomb, 1982). Therefore, at moderate induction levels, it would be desirable to enhance flower bud induction for alternate bearing cultivars during the winter of the ON-year to increase yield in the OFF-year.

Enhancement of flower bud induction may be achieved by applying foliar low-biuret urea or PO_3 (Table 4-4). Manipulation of foliar nitrogen content (by means of applying foliar urea) enhances floral intensity by improving the conditions that convert vegetative to reproductive buds (Ali and Lovatt, 1995). The urea action may be a stress response from the rapid release of ammonia in the plant tissue (Albrigo, 1998a). Increased nitrogen levels may serve to initiate the flowering process (Lovatt et al., 1988). During the induction period, increases in PO_3 levels also are considered to enhance flower bud induction (Albrigo, 1998a; Parra and Ortuño, 1981). The cultivars for which enhancement of flower bud induction is recommended include all grapefruit and

mandarins approaching the OFF year, 'Valencia' and 'Hamlin' sweet oranges also in the OFF year. For maximum absorption it is recommended to spray preferably during warm conditions (days with maximum temperatures above 20°C) avoiding cold conditions (days with maximum temperatures below 15°C).

3) Reduction of induction levels. During upper moderate or high induction levels it is often desirable to reduce flower bud induction (reduce flower formation) for alternate bearing cultivars ('Minneola,' navels, 'Ambersweet,' 'Valencia,' 'Hamlin,' and mandarins) approaching the ON year. Reduction of induction levels may be achieved through the foliar application of gibberellic acid (GA₃) (Table 4-5). Gibberellins can significantly reduce the number of flowering buds (Monselise and Halevy, 1964).

The expert system recommends the foliar application of GA₃ during warm conditions for maximum absorption (avoiding periods of low temperatures). Applications of GA₃ may be delayed if the trees are under water stress. The effect of a GA₃ application is diminished after 2 to 3 d of the warm conditions. The effect of the application 5 to 7 d after or into the warm conditions is minimum.

In addition, reduction of induction levels may be desirable in situations when the induction stimulus has been excessive to reduce the level of floral intensity. The expert system recommends the foliar application of GA₃ to reduce the number of flowering buds (Table 4-7).

4) Management of secondary or extended flowering. After a moderate level of induction has been reached, the chance of a secondary bloom may be reduced by enhancing floral induction of the first (primary) flowering using foliar low-biuret urea or PO₃ (Table 4-6). This is the same strategy as when enhancing flowering when an OFF-

year is anticipated. Again the spray should be applied during warm conditions (days with maximum temperatures above 20°C) to maximize absorption, but before initiation of flower buds has proceeded beyond 2 or 3 days. Spray may be delayed, if trees are under water stress, until just before irrigation or rain releases the buds for growth. The cultivars for which this strategy is recommended include 'Valencia,' 'Hamlin,' grapefruits, and mandarins in the OFF year.

Transition from Induction to Differentiation Models

Based on the characterization of historical weather records, a number of expert system rules were developed to determine the transitions of floral induction to the initiation of differentiation. This transition was determined based on floral induction status and the presence of warm conditions. The warm conditions required to initiate differentiation of flower buds are time and temperature dependent on the level of induction at the time of the warm conditions.

Models for Floral Organ Differentiation – Time to Bloom Models

The time to bloom models consist of two equations that estimate time to peak flowering and a weather data acquisition system that collects weather data on a real-time basis. Both models estimate days to full bloom based on phenological development as a function of temperatures after fulfillment of induction stimuli and conditions for initiation had occurred (warm conditions trigger) (Chapter 3). Each model was developed for a different geographical area. The model based on degree-day analysis should be adequate for most Florida citrus production areas, but may not be used in other production regions (tropical or Mediterranean). As the equation for floral development models the actual physiological process, it should yield reasonable results in any production area. If improved this equation must hold universal value in all citrus production areas.

However, this equation needs to be improved before any commercial use in any production area.

Once initiation was determined to have occurred both models use a weather data acquisition system to collect the occurring temperatures in a real-time basis. Both models estimate percentage of floral development based on local weather data. Since one of the objectives of the models is to predict flowering date, it is necessary to estimate ambient temperatures to the expected flowering date. Due to the uncertainty associated with weather variability, use of historical average hourly and daily temperatures for the complete prediction of flowering date is questionable.

Two basic approaches are followed to estimate ambient temperatures to the expected flowering date. Long-term weather forecasts are automatically obtained from the NOAA Weather Service Website and used for the prediction of ambient temperatures of the first 7-8 d. The rest of the temperatures that complete the differentiation period are obtained from average temperatures of the location. In the southeastern US, El Niño-Southern Oscillation (ENSO) can influence crop phenology through its interactions with weather. El Niño years tend to be cooler and wetter, and La Niña years tend to be warmer and dryer than neutral years during fall and spring (Table 4-8) (Hansen et al., 1998). Hourly and daily temperature averages were obtained for neutral, El Niño and La Niña years. The user can choose the average temperatures according to the status of ENSO cycle.

At any point during the differentiation period, the program is able to estimate the time to peak anthesis based on the 7-8 d forecast and average hourly temperatures. The

prediction of the date of peak anthesis is adjusted dynamically as actual temperatures occur and replace both the forecasted and average temperatures.

Discussion and Conclusions

Whereas the developed parameters for induction monitoring and the rules for the transition from induction to differentiation seem to yield good results, better understanding of crop behavior is still needed. This model provides the guidelines for more specific research towards a better understanding of onset of differentiation.

The DiSC-DSS estimates flowering date using two different models. The first model uses average daily temperature and physiological parameters to determine percent of floral development. The second model is a statistical relationship developed for Florida conditions. The second model has a better fit than the first. However, the first model should provide an accurate estimate for other citrus production regions. If the model is to be used in Florida, the second model should be more accurate and should provide better estimations for the prediction of citrus flowering. More detailed research information on floral development rates should provide better floral development factors for more accurate estimation based on physiological parameters.

All models are subjective constructions aiming to provide either a better understanding of crop behavior or a prediction of it (Passioura, 1974). Although the developed phenology models for flower bud induction and flower bud development still need improvements, they appear to be reasonable first versions for validation under field conditions with grower cooperators.

The overall framework for the DSS integrates general grove setup with record keeping and the ability to apply expert systems to the developing tree and crop each year.

Record keeping also allows retention of previous records of phenology and production practices for each block for several years. The DISC-DSS also provides a flexible structure to incorporate more expert systems, and information as they become available through research. The Decision Support System also provides the ability to manage flowering of citrus in Florida. Until recently, management of citrus flowering was not possible due to lack of understanding of the process under Florida conditions and the lack of tested procedures to alter flowering intensity. The DSS joins the knowledge of the flowering process as related to climate with management tools and now allows Florida citrus growers to understand and manage the flowering process to some degree. The overall DSS gives Florida citrus growers the ability to keep an overview of all production practices and apply them in a timely manner with prior knowledge of upcoming phenological events.

Table 4-1. Induction monitoring expert system rule database set for the determination of induction status

Induction level	Inductive conditions [‡]	Description of induction level	Weather conditions needed for initiation
Low	0-700 h below 20°C	Induction stimuli is not sufficient to cause economic crop levels of floral organ differentiation	No economically significant initiation is expected
Moderate	700-1000 h below 20°C	Induction stimuli is sufficient that economic levels of buds would be expected to initiate and develop flowers if warm conditions occur	700-850 h below 20°C. Twelve days with maximum temperatures above 25°C with no more than 2 intervening days below 20°C 850-1000 h below 20°C. Seven days with maximum temperatures above 20°C, with no more than three intervening days below 20°C
High	1000-1200 h below 20°C	Warm conditions with sufficient soil moisture will initiate enough differentiation that no additional flowering is likely	7 days with maximum temperatures above 20°C with no more than two intervening days below 20°C
Excessive	Above 1200 h below 20°C	If initiation has not occurred, excessive flowering would be likely to occur	5 consecutive days with maximum temperatures above 20°C with no more than 4 intervening days below 20°C

[‡] Inductive conditions refer to the number of hours below 20°C from 1 Oct. to the current date

Table 4-2. Expert System rule database set to delay the initiation of differentiation at low induction levels

Induction level	Inductive conditions	Rules	Output text
Low (low end)	0-300 hours below 20°C	Tree age: ≥ 4 Cultivars: All Soil type: shallow depth Warm conditions: Y/N	Management of flower bud induction is limited during this low induction level. Sufficient warm conditions will cause buds to sprout into vegetative growth.
Low (low end)	0-300 hours below 20°C	Tree age: ≥ 4 Cultivars: All Soil type: deep sands Warm condition: Y/N	Management of flower bud induction is limited during this low induction level. Presence of warm conditions will not cause buds to sprout into either vegetative or reproductive growth.
Low (high end)	300-700 hours below 20°C	Tree age: ≥ 4 Cultivars: All Soil type: shallow depth or bedded Warm conditions: y/n	The induction process is not complete and bud sprouting at this stage has the potential of leading to multiple blooms. The best way to manage the possibility of multiple blooms is by withdrawing irrigation.
		Warm conditions: no Warm conditions: yes	Maintain low soil water levels Current warm conditions may cause some buds to sprout and develop into flowers. It is recommended that trees should be water stressed by irrigation withdrawal. Due to the nature of your soil type, sufficient water stress may be achieved in 4 to 5 days
Low (high end)	300-700 hours below 20°C	Tree age: ≥ 4 Cultivars: All Soil type: deep sands Warm conditions: y/n	At this point in time, the induction process is not complete and bud sprouting has the potential of leading to multiple blooms. The best way to manage the possibility of multiple blooms is by withdrawing irrigation.
		Warm conditions: no Warm conditions: yes	Maintain low soil water levels. This is particularly important in deep sands as achieving water stress may require a period of two to three weeks after irrigation is stopped.
			Warm conditions may cause some buds to sprout and develop into flowers. Adequate irrigation management should have started one to two weeks ago. Water stress should be well underway to diminish the possibility of multiple blooms.

Table 4-3. Expert System rule database set to delay the initiation of differentiation at moderate induction levels

Induction level	Inductive conditions	Rules	Output text
Early moderate induction levels	700-700 hours below 20°C	Tree age: ≥ 4 Cultivars: All Soil type: all Warm conditions: y/n	Onset of differentiation may be manageable at this stage through water stress Presence of warm conditions will trigger differentiation before obtaining full induction levels, which may result in multiple blooms. Delaying the onset of differentiation may be achieved by reduced soil water availability by irrigation withdrawal.
Early moderate induction levels	700-700 hours below 20°C	Recommended cultivars: All Soil type: shallow depth soils or bedded groves Cultivars: Hamlin or Pineapple	Since the grove is on bedded groves or shallow soil, water stress may be achieved in three to four days. Water stress should be approached carefully in certain cultivars such as Hamlin or Pineapple as it may cause some fruit drop. Due to increased resistance to fruit drop Valencia can withstand water stress more readily than other cultivars
Early moderate induction levels	700-700 hours below 20°C	Cultivars: Valencia Recommended cultivars: All Soil type: deep sands Cultivars: Hamlin or Pineapple Cultivars: Valencia	Since the grove is on deep soils, sufficient water stress may be achieved in 2 to 3 weeks. Water stress should be approached carefully in certain cultivars such as Hamlin or Pineapple as it may cause some fruit drop. Valencia can withstand water stress more readily than other cultivars

Table 4-4. Expert System rule database for the enhancement of flower bud induction

Induction level	Inductive conditions	Rules	Output text
Moderate	700-1000 hours below 20°C	Tree age: > 4 Recommended cultivars: Valencia, Hamlin, grapefruit, alternate bearers mandarins in the off year Soil type: all	Enhancement of flower bud induction may be achieved by applying urea or PO_3 . To maximize foliar uptake, spray should not be applied during cold conditions (below 10°C) If floral initiation is occurring, avoid more than two days into warm conditions. Spray may be delayed if trees are under water stress Application rates: 25-28 pounds of N/acre as urea. Spray volumes 25-125 gallons ULV speed spray 50-60 gts at 4 miles per hour

Table 4-5. Expert System rule database for the reduction of induction levels

Induction level	Inductive conditions	Rules	Output text
Moderate	700-1000 hours below 20°C	<p>Minimum tree age: ≥ 4</p> <p>Recommended cultivars: Heavy blooming cultivars with poor set (seedless) such as Minneola, Navels, Ambersweet, alternate bearer cultivars into the ON year (mandarins)</p>	<p>Reduction of induction levels may be achieved through the application of GA (GA_3 or proligibb)</p> <p>Spray should preferably be applied during moderately warm conditions. It may be delayed if trees are under water stress</p> <p>Application rates: 20 ounces of ProGIBB (4%) per acre applied at 125-175 gallons per acre with 0.05% Silwet L-77. Lower rates, gallons per acre and surfactants may be effective but have not been tested.</p> <p>At 150 gallons/acre, this would be : 13.3 ounces of GA (4%) and 6.3 ounces of Silwet L-77 per 100 gallons.</p> <p>Some leaves may be knocked off of trees, especially where greasy spot has damaged foliage or temperatures are high at the time of application, however we have seen increased cropping even after some leaf loss. Fruit still present on trees may re-green. Bloom is likely to be reduced, will be associated with more leafy flush, and may be earlier or later and more concentrated than in untreated trees. This is an experimental procedure, however it has been found that final crop has been markedly increased by most treatments</p> <p>There is a warm condition in the forecast. Application of GA (GA_3) may be scheduled to take advantage of greater absorption during this time</p> <p>No warm conditions are in the forecast. Avoid applying GA_3 during cold weather</p> <p>The effect of the application of GA_3 is diminished after 2-3 days of the warm conditions. Although the effect of the application 5-7 days after or into the warm conditions is minimum. There is evidence that the application may still be effective 7 days into or past the warm spell</p>
		Warm conditions: Y	
		Warm conditions: N	
		Warm conditions: In the past	

Table 4-6. Expert System rule database to decrease the intensity of secondary blooms

Induction level	Inductive conditions	Rules	Output text
Moderate	700-1000 hours below 20°C	<p>Minimum tree age: ≥ 4 years old</p> <p>Recommended cultivars: Valencia, Hamlin, grapefruit, alternate bearers mandarins in the off year</p> <p>Warm conditions: Y</p> <p>Warm conditions: N</p> <p>Warm conditions: > 5 days in the past</p>	<p>Sprays of urea or PO_3 before the onset of differentiation may decrease intensity of secondary blooms</p> <p>Spray should preferably be applied during a moderately warm conditions (avoid cold conditions)</p> <p>Spray may be delayed if trees are under water stress</p> <p>Application rates: 25-28 pounds of N/acre as urea. Spray volumes 25-125 gallons ULV speed spray 50-60 gpa at 4 miles per hour</p> <p>There are warm conditions in the forecast. Application of urea or PO_3 may be scheduled to take advantage of greater absorption during this time</p> <p>Do not apply urea or PO_3 during low temperature conditions</p> <p>The effect of the application of urea or PO_3 is diminished after 2-3 days of the warm conditions. The effect of the application 5-7 days after or into the warm conditions is minimum.</p>

Table 4-7. Expert System rule database to reduce induction levels and decrease flowering intensity

Induction level	Inductive conditions	Rules	Output text
Moderate	> 1200 hours below 20°C	<p>Minimum tree age: >=4 years old</p> <p>Recommended cultivars: Valencia, Hamlin, grapefruit, alternate bearers mandarins in the off year</p>	<p>Reduction of induction levels may be achieved through the application of GA (GA₃ or proggibb)</p> <p>Spray should preferably be applied during moderately warm conditions. It may be delayed if trees are under water stress</p> <p>Application rates: 20 ounces of ProGHB (4%) per acre applied at 125-175 gallons per acre with 0.05% Silwet L-77. Lower rates, gallons per acre and surfactants may be effective but have not been tested.</p> <p>At 150 gallons/acre, this would be : 13.3 ounces of GA (4%) and 6.3 ounces of Silwet L-77 per 100 gallons.</p> <p>Some leaves may be knocked off of trees, especially where greasy spot has damaged foliage or temperatures are high at the time of application, however we have seen increased cropping even after some leaf loss. Fruit still present on trees may re-green. Bloom is likely to be reduced, will be associated with more leafy flush, and may be earlier or later and more concentrated than in untreated trees. This is and experimental procedure, however it has been found that final crop has been markedly increased by most treatments</p> <p>There is a warm condition in the forecast. Application of GA (GA₃) may be scheduled to take advantage of greater absorption during this time</p> <p>No warm conditions are in the forecast. Avoid applying GA₃ during cold weather</p> <p>Initiation is likely to have already occurred.</p> <p>The effect of the application of GA₃ is diminished after 2-3 days of the warm conditions. Although the effect of the application 5-7 days after or into the warm conditions is minimum. There is evidence that the application may still be effective 7 days into or past the warm spell</p>
		Warm conditions: Y	
		Warm conditions: N	
		Warm conditions: > 5 days in the past	

Table 4-8. Summary of El Niño and La Niña years from 1958-1959 to 1999-2000. Years not mentioned are considered neutral

El Niño Year	La Niña Year
1963-64	1964-65
1965-66	1967-68
1969-70	1970-71
1972-73	1971-72
1976-77	1973-74
1982-83	1975-76
1986-87	1988-89
1987-88	
1991-92	

APPENDIX A
DEVELOPMENT OF FLOWERING BUDS IN EXCISED STEMS OF SWEET
ORANGE [*Citrus sinensis*, (L). Osbeck]

Deciduous trees enter dormancy and develop cold hardiness to survive severe winter conditions. This type of dormancy is called endodormancy as the bud itself controls dormancy release (which contrasts with ecodormancy in which the release of dormancy is controlled by environmental factors). Exposure of the bud to low temperatures is needed for the release of endodormancy (Lang et al., 1987). The characterization of temperatures needed to break endodormancy is referred to as the chilling requirement (Weinberger, 1950). Buds that have completed their chilling requirement go into ecodormancy. Ecodormant buds on cut shoots respond and swell at room temperature. This feature is commonly used in dormancy studies to determine whether or not the chilling requirement has been satisfied (Couvillon and Erez, 1985; Gardea et al., 1994; Jian et al., 1997).

Citrus are evergreen trees that do not go into endodormancy. However, if grown under subtropical conditions, low temperature stress during the wintertime induces many vegetative buds to become flower buds. The subsequent release of this stress by higher temperatures during springtime signals the beginning of reproductive growth of the buds and brings about floral organ differentiation (Cassin et al., 1969).

Monitoring the effect of low temperatures on flower bud induction of citrus trees during the wintertime presents a number of problems. Most importantly, it is difficult to manipulate the low-temperature stress. Temperature control is unpractical for the

majority of the situations due primarily to the large size of nonjuvenile citrus trees. With the exception of growth chambers, the only available method that has been developed for induction monitoring is the girdling and defoliation technique. This method is based on an early assumption that the induction stimulus is perceived by the leaves, and is translocated through the phloem. Therefore, after girdling and defoliation no inducing stimulus can reach the buds distally to the girdle. The girdling and defoliation method also assumes that girdling does not affect the percentage of sprouting buds (Ayalon and Monselise, 1960; Furr and Armstrong, 1956; Furr et al., 1947). Since those early studies, a number of drawbacks have been identified to the girdling and defoliation technique. Although the presence of leaves does have a direct role during the induction period, in vitro studies of inductive conditions proved that the induction stimulus could also be perceived at the bud itself (García-Luis et al., 1992; Tisserat et al., 1990). Girdling (without defoliation) tends to increase flowering by changes in carbohydrate levels distal to the girdle and by the effects on hormonal levels (Erner, 1988; Wallerstein et al., 1973). Despite the common use of the girdling and defoliation technique, apparently no study has analyzed the hormonal and/or carbohydrates changes following girdling and defoliation.

There is a need for an accurate method to monitor induction through the winter period as the traditional girdling and defoliation presents several drawbacks. Based on experiences with deciduous trees, it was deemed possible to develop a technique to monitor induction by forcing buds on cut shoots to express their reproductive or vegetative potential.

Early attempts to culture citrus buds *in vitro* resulted in the formation of vegetative shoots exclusively (Altman and Goren, 1974; Guardiola et al., 1982). The first successful attempt reported to produce flowers *in vitro* was performed on resting buds cultured in a medium supplemented with sucrose and a cytokinin (García-Luis et al., 1989). Since then a number of *in vitro* studies of buds have been performed. The response of buds in tissue culture conditions is affected by the same factors that affect the *in vivo* response. The need for the induction stimuli was observed as no flowers formed in buds cultured during summer and early fall. Low-temperature exposure (15/10°C) increased the ability of the buds to sprout *in vitro* (García-Luís and Kanduser, 1995). The presence of fruit *in vivo* reduces the flowering response of *in vitro* buds to chilling. This direct effect of fruit on the buds persists even after fruit removal (García-Luís and Kanduser, 1995). Ringing, which enhances flowering *in vivo*, increases the number of explants flowering *in vitro* as well. There are, however, reported differences: the percentage of flowering explants was found to be lower than the percentage of naturally flowering buds in spring, (García-Luis et al., 1989). *In vitro* culture of citrus buds may have different low-temperature requirements to induce flowering (Tisserat et al., 1990).

The objectives of this study were to determine if buds might be forced to grow and develop into flowers once the "low temperature induction requirement" has been satisfied, and to determine whether this technique can be used to monitor floral bud induction of citrus trees.

Materials and Methods

Experiment 1. Preliminary Studies

The first experiment was conducted as a preliminary study to determine the feasibility of the study and to identify problems in the development of the technique. Preliminary studies consisted of the collection of shoots in the winter time, surface sterilization and forcing them to grow in distilled water at different temperatures, under low to moderate illumination conditions. Three sets of 8 shoots of mature trees of 'Hamlin' sweet orange [*Citrus sinensis*, (L). Osb.] (at least 10 cm long) were collected during winter inductive conditions from mature field trees located at the Citrus Research and Education Center in Lake Alfred, Florida. Leaves and thorns were removed before shoot collection. Shoots were collected using fruit clippers. Shoots were washed and surface sterilized and placed inside of mason jars with distilled water and sterilized sand at their bottom. Jars were covered using transparent polyethylene with holes. Jars were then placed at 20, 25, and 30°C, under high illumination conditions.

Experiment 2. Forcing Technique for the Development of Flowers in Previously Induced Buds.

Plant materials consisted of two sets of eight shoots of mature trees of sweet orange that had been induced to flower using low-temperature inductive conditions naturally occurring during the winter of 1998-1999. The study was repeated using eight shoots of containerized sweet orange trees that had been induced to flower by exposing the trees to low-temperature stress (10°C) for a period of 3 weeks and to water stress for two additional weeks.

The forcing technique can be divided into collection of plant materials, shoot preparation, and forcing.

Collection of plant materials

To collect plant materials, shoots of different ages were defoliated and their thorns completely removed, being careful not to damage the bud. Stems were detached using fruit clippers or wire cutters. Shoot cuts were made inside a solution containing 200 mgL⁻¹ hydroxyquinoline sulfate to minimize vascular blockage (van Doorn and Perik, 1990). Shoots were collected in the field and placed inside plastic bags with 200-mgL⁻¹ hydroxyquinoline sulfate to minimize stem dehydration and subsequent mortality.

Shoot preparation

Shoots were surface-sterilized for 30 s in a sodium hypochlorite (Chlorox) solution (10% v v⁻¹), rinsed, and dried. Once dried, the shoots were covered with Carnuba-based wax (amended with 2000-mgL⁻¹ Imazalil and 500-mgL⁻¹ Thiabendazole or TBZ). Amendments were added to minimize fungal and bacterial activity.

Once the wax had dried, the base of each stem (at least 0.5 cm) was re-cut holding at least 2 cm of the stem under distilled water. The stems were then placed inside transparent glass containers with sand at the bottom (for mechanical support). Sand had been previously sterilized by autoclaving or by soaking it in a 1% sodium hypochlorite solution. Once in the container, the sand was saturated with solutions containing 0.2% sucrose and 200-mgL⁻¹ hydroxyquinoline sulfate. Treatments included cytokinin (kinetin) solutions at 0.1, 1, 10, and 100-mgL⁻¹. The containers were then covered with transparent polyethylene. Holes were punched at about 10 x 10 cm intervals for air exchange.

Shoot forcing

The shoots were forced to grow at three different temperatures (21, 25, or 31°C).

Results

Experiment 1. Preliminary Studies

During the preliminary studies, a number of problems were identified. Although shoot mortality was low, some shoots died, probably due to dehydration or vascular blockage. Surface decay problems, due primarily to fungal contamination, were also observed. Fungal growth was present mainly in a position axillary to the buds, particularly in leftover structures that were part of leaves and thorns. Another identified problem was the fact that the sprouting buds developed vegetative growth exclusively. Vegetative growth was obtained regardless of the induction level to which the buds had been exposed before forcing. In all situations, growth obtained from sprouting buds was small and detached easily from the stem. After forcing for 2 weeks, the growth obtained from the buds collapsed.

Experiment 2. Forcing Technique for the Development of Flowers in Previously Induced Buds.

In the absence of cytokinins, vegetative growth developed in all the sprouting buds regardless of their induction level. Flowers developed on flower-induced buds forced to grow in 1 and 10 mgL⁻¹ cytokinins. Greater cytokinin levels inhibited the development of functional growth. Table 4-1 summarizes the observed response of citrus buds to different concentrations of cytokinins and sucrose.

Discussion

The majority of the problems that were encountered in preliminary studies were solved by using greater care to prevent contamination and by using the additional solutions. A concentration of 2% sucrose qualitatively increased the response in growth from the buds. Decay problems due to bacterial or fungal contamination were minimized

by carefully removing the entire thorn, petiole, and leaf structures; by surface-sterilization of the shoots; and by the addition of carnauba wax amended with imazalil and TBZ. The potential for physiological blockage of the vascular systems was diminished by the direct effect of hydroxyquinoline on bacteria and fungi, or indirectly by its effect on the pH of the forcing solution (Marousky, 1969; van Doorn and Perik, 1990).

Reproductive growth was seen on a number of induced shoots and depended on the concentration of cytokinins in the solution. Flower development in excised shoots depended primarily on whether or not the bud had previously been induced to flower and on the presence of cytokinins in the forcing solution. If the buds were forced to grow exclusively in a solution containing 0 and 0.1 mgL⁻¹ cytokinins, essentially no flowers developed. Some flower development occurred in the presence of solutions at 1 and 10-mgL⁻¹ solution of cytokinins. Higher cytokinin concentrations (100-mgL⁻¹) resulted in the sprouting of numerous adventitious buds, with little growth of each. No distinction was possible on the vegetative or reproductive nature of the new growth. As cytokinins are synthesized in growing roots and are translocated through the xylem to the developing buds (Saidha et al., 1983), a more active role of growing roots in flower development is inferred. As girdling and defoliation eliminates phloem passage of hormones and carbohydrates, this study supports the concept that cytokinins, possibly from the roots, are required for complete flower bud development.

Other remaining problems are related to the fragility and early collapse of the forced new growth. The new growth, whether vegetative or reproductive, was very fragile and detached easily from the shoots. The maximum length of time that the buds

were able to remain in forcing conditions was 2 weeks. At this time physiological collapse occurred and the forced growth collapsed and died. Two weeks was deemed sufficient to determine whether or not the bud had made the transition from vegetative into reproductive stages indicating that low temperature induction had been satisfied.

However, whenever reproductive growth appeared, only one flower per bud was observed. In Florida, under similar induction levels, buds in intact shoots under normal differentiation conditions develop more than one flower per bud. Further research should be focused on obtaining growth that more accurately depicts the flowering behavior of citrus trees at similar induction levels.

Conclusions

The majority of the identified problems during the preliminary studies to force flower buds were resolved. Decay problems were solved by carefully removing leftover structures, and by applying fungicides to the wax. Vascular blockage was prevented with the use of hydroxyquinoline sulfate. Growth response was enhanced with the addition of sucrose into the forcing solution. The development of reproductive structures in addition to vegetative structures was possible with the addition of cytokinins.

The developed technique proved useful for the expression of the reproductive or vegetative nature of the forced buds. Buds forced to grow showed different phenological responses comparable to the flowering behavior of buds of intact shoots. Whereas development of reproductive organs occurred on flower-induced buds (forced in a solution with 1 or 10 mgL⁻¹ of cytokinin levels), vegetative growth occurred (no flowers developed) on noninduced buds on excised shoots forced to grow at any levels of cytokinins.

Although in this study it was determined that flower-induced buds might be forced to grow and develop into reproductive structures, it is still not possible to use this technique to monitor floral bud induction of citrus trees. Additional research is necessary to determine the conditions that will lead to the full expression of the reproductive potential of induced buds (cytokinin requirements and conditions needed to extend growth time before the physiological collapse of the forced growth). Once these conditions are determined, further research should focus on quantitatively evaluate the response of the forced buds at different induction levels in comparison with buds growing on intact shoots in trees. If comparable reproductive response is obtained, this technique may prove to be a valuable replacement for the girdling and defoliation technique, as the bud forcing technique is simple, inexpensive, and manageable.

Table A-1. Summary of observed response of citrus buds with two different levels of floral induction to different concentrations of cytokinins and sucrose

Cytokinin Concentration (mg L ⁻¹)	Sucrose Concentration (%)	Induced bud	Observations
0	0	Yes	Vegetative growth exclusively
0	2	Yes	Vegetative growth exclusively Greater growth was obtained in comparison with the first treatment
0.1	2	Yes	Vegetative growth exclusively
1	2	No	Vegetative growth exclusively
1	2	Yes	Flowers developed on the first and second replications
10	2	Yes	Flowers developed on the second replications (1 flower per bud)
100	2	Yes	No flowers developed Presence of numerous adventitious buds No vegetative growth

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BIOGRAPHICAL SKETCH


Mr. Juan Ignacio Valiente Banuet was born in Mexico City, Mexico on December 27th, 1967. He was the seventh of eight children born to Guadalupe Banuet and Camilo Valiente. At 17, he moved to Monterrey, N.L. (Mexico) where he attended the Monterrey Institute of Technology [*Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM)*]. At ITESM, he majored in Agronomical Engineering (specializing in Field Production). One semester before his graduation he came to the University of Florida, for the first time, to work on his Bachelor of Science (B.S.) thesis with Dr. Fedro S. Zazueta. After this 6-month stay he returned to Monterrey N.L. to complete his B.S. degree.

After he obtained his B.S. degree from ITESM he started his Master of Science degree (M.S.) in Horticultural Sciences at the University of Florida in Gainesville, Florida. Upon obtaining his M.S. degree, and after a short stay in Gainesville, he returned to his native Mexico and obtained a job at the Trustfunds for Agriculture of the Central Bank of Mexico [*Fideicomisos Instituidos en Relación con la Agricultura (FIRA)*] in Morelia, Mich. At the end of 1996 he decided to continue his education and pursue the Ph.D. degree, which he started in January 1997.


The first 8 months of his Ph.D. program he worked on a turf-grass project under the direct supervision of Dr. Zazueta. He was later incorporated into the Decision Information Systems for Citrus (DISC) project. The DISC project is being developed by a multidisciplinary group that focuses on implementing the most recent advances in

computer technology for citrus production. He worked on this project as part of his Ph.D. program under the direct supervision of Dr. Gene Albrigo. During his Ph.D. studies he was an active member of the Mexicans in Gainesville student organization (of which he was president from 1998 to 1999), and the Citrus Club (of which he was president from 2000-2001).

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


James J. Ferguson, Chairman
Professor of Horticultural Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Howard W. Beck, Cochairman
Associate Professor of Agricultural and
Biological Engineering

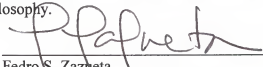
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


L. Gene Albrigo
Professor of Horticultural Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

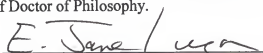

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Professor of Horticultural Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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